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Award Number: DAMD17-98-1-8120

TITLE: Psycho-Endocrine-Immune Profile: Implications for  
Quality of Life in Breast Cancer Patients

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REPORT DATE: November 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

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20030520 083

**REPORT DOCUMENTATION PAGE**Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY (Leave blank)</b>		<b>2. REPORT DATE</b> November 2002	<b>3. REPORT TYPE AND DATES COVERED</b> Final (1 Nov 98 - 31 Oct 02)	
<b>4. TITLE AND SUBTITLE</b> Psycho-Endocrine-Immune Profile: Implications for Quality of Life in Breast Cancer Patients			<b>5. FUNDING NUMBERS</b> DAMD17-98-1-8120	
<b>6. AUTHOR(S) :</b> Linda J. Janusek, R.N., Ph.D.				
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  Loyola University of Chicago Maywood, Illinois 60153  E-Mail: <a href="mailto:ljanuse@luc.edu">ljanuse@luc.edu</a>			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b>				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited				<b>12b. DISTRIBUTION CODE</b>
<b>13. ABSTRACT (Maximum 200 Words)</b>  Biopsy of the breast for cancer diagnosis is an emotional experience, characterized by anxiety and fear. This experience may impair immune responses. This study evaluated a woman's immunological and psychological response pre and post breast biopsy. Perceived stress, anxiety, and mood disturbance were heightened pre-biopsy. Post-biopsy; perceived stress, anxiety, and mood disturbance decreased but did not return to levels reported by non-biopsied control women. Natural killer (NK) cell activity was significantly depressed pre and post biopsy, when compared to non-biopsied, control women. Post biopsy, NK cell activity was less than that exhibited pre biopsy. No changes in number of NK cells were observed at either pre or post biopsy time points. Production of INF $\gamma$ was significantly reduced pre and post biopsy and production of IL-4, IL-6, and IL-10 were significantly increased by the experience of breast biopsy. The reduction in NK cell activity after breast biopsy was more marked in women with malignant breast biopsy findings. The results also suggest that women who report the highest levels of stress or mood disturbance have the most marked changes in immune function. Thus, impending breast biopsy is marked by increased perceived stress, anxiety, and mood disturbance, which is relieved post-biopsy, but does not return to levels reported by non-biopsied women. In conclusion, stress-induced alterations in immunity are not transient but persist beyond the acute experience of the biopsy. This may be of particular relevance to women diagnosed with malignancy since they will be facing additional stressors related to cancer treatment and adaptation to illness.				
<b>14. SUBJECT TERMS</b> breast cancer, psychological stress, mood disturbance, breast biopsy, human peripheral mononuclear cells, natural killer cells, IL-2, IL-4, IL-6, IL-10, interferon				<b>15. NUMBER OF PAGES</b> 83
				<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

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## (1) INTRODUCTION

A large body of evidence supports the assertion that stressful life experiences and negative emotions precipitate immune dysregulation, which may influence health outcomes (1-3, 5-9, 20). Stress perception and mood disturbance, which occur in response to stressful events, have been demonstrated to depress the immune system (11, 22, 24). Abundant evidence supports an interactive relationship among stress and homeostatic immune function (11, 27, 38). The results reported herein demonstrate that women undergoing breast biopsy experience psychological stress that begins early, during the diagnostic phase of the disease. These results are consistent with those of others who have demonstrated increased psychological stress to predict changes in immune function in women with breast cancer (8). This may be particularly important because the effects of stress have been shown to be upon immune effector cells that participate in cancer control (10, 12, 26, 32, 36). Fresh lines of evidence demonstrate these cells to contribute to protection from tumors (13, 19, 28, 30) including tumor initiation (29, 33), primary tumor growth (33), and tumor metastasis (29, 33). Over one and a half million women undergo breast biopsy annually in the USA (34). Breast cancer diagnosis is a time of considerable uncertainty, anxiety, and emotional distress (14, 25). This emotional experience often begins with the discovery of clinical findings that indicate the need for biopsy of the breast. Women awaiting breast biopsy report higher levels of stress compared to patients awaiting general surgery (18). Reciprocal neuro-chemical pathways and shared receptor systems connect the nervous, endocrine, and immune systems (21, 35). This intricate communication network provides the link whereby perceived environmental stressors may effect the immune system and influence the course of disease (37). Numerous research studies have documented the impact of psychosocial stress on the human immune response (17). Several of these studies have examined the relationship between stress and natural killer cell activity (NKCA) and these studies have shown that stress can influence NKCA (32). Andersen *et al.* (8) studied the stress-immune response of women within four months of their breast cancer surgery but prior to adjuvant therapy initiation. The results of that study indicated that higher stress levels were predictive of lower NKCA, diminished natural killer (NK) cell response to interferon (IFN) gamma, and decreased lymphocyte proliferation (8). It is possible that stress may influence cancer control. Although a direct relationship between NKCA and cancer has not been equivocally established, patients with a variety of solid tumors (e.g., breast, cervix, endometrium, ovary, lung) do exhibit reduced NKCA (26). The effects of stress upon the immune system extend not just to NK cells but also to the production of cytokines. Heightened levels of stress are related to decreased synthesis of IFN  $\gamma$  (16) and a poorer NK response to IFN  $\gamma$  and interleukin-2 (IL-2) has been observed in stressed individuals compared to non-stressed individuals (15). Others have reported that posttraumatic stress disorder (23) and academic exam stress (24) also lead to cytokine dysregulation. Cytokine dysregulation in response to stress alters Th1/Th2 cytokine balance, leading to decreased production of IFN gamma (a Th1 cytokine) and enhanced production of IL-4 (a Th2 cytokine) (4). Such a change in cytokine balance can depress NK cell function (31). The purpose of this study was to investigate psychological stress and its' potential impact on the immune response (NKCA and cytokine balance) of women before and after breast biopsy. The design of this study allows the exploration of the biological links between stress and immune function at an early point in a woman's encounter with cancer.

## (2) BODY

**Methods** - All women over 18 years of age, who sought consultation at the Breast Care Center of Loyola University's Cancer Center and who subsequently received a breast biopsy (excluding fine needle aspirates) were eligible subjects. Exclusionary criteria included: pregnancy, prior (within 5 years) or current history of cancer, recent history of major psychiatric disorder or concurrent major immune-based disease, and active substance abuse. Women were studied at the following four time points:

- T<sub>1</sub> - Initial consultation at the Breast Care Center
- T<sub>2</sub> - Day of biopsy, prior to the actual biopsy
- T<sub>3</sub> - At return clinic visit, 10-14 days after notification of biopsy results
- T<sub>4</sub> - 1-2 months after T<sub>3</sub>.

At each time period volunteers completed informed consent documents, had their blood drawn, and completed the psychological measures and a health history questionnaire.

**Psychological Measures:** Psychological stress was defined as an individual's response to perturbations from the environment (i.e., breast biopsy and possible cancer diagnosis). The psychological measures included a visual analogue scale (VAS) for global stress and for biopsy-related stress, Cohen's Perceived Stressor Scale (PSS), Spielberger's State Trait Anxiety Inventory (STAI), the Profile of Mood States (POMS), Sense of Coherence, and Resilience.

**Laboratory Measures:** Peripheral blood mononuclear cells (PBMC) were derived immediately by Ficoll/Hypaque separation. These isolated PBMC's were assessed for NKCA against [<sup>51</sup>Cr] labeled K562 tumor targets as described previously (20). Cytokine production was measured by stimulation of peripheral blood mononuclear cells in bulk culture. Culture supernatants were collected after 48 hrs and cytokines were measured using standard ELISA kits (R & D Systems, Minneapolis, MN). Plasma cortisol and DHEA-SO<sub>4</sub> were measured using radioimmunoassay kits from Diagnostic Products Corporation.

### Statement of Work To Date.

**Task 1.** Determine the psycho-endocrine and NK cell response of women to the experience of breast biopsy for cancer diagnosis (months 1-48). The total number of women enrolled in this study was 138. Of the total women enrolled in this study, 17 women did not have a biopsy performed. This was often a clinical decision occurring on the day of biopsy prior to the actual procedure. One woman had a cyst removed, which was not sent to pathology. Six women withdrew from the study. Of the 115 women undergoing breast biopsy, 83 had benign biopsy results (72%) while 32 women were diagnosed with malignancy (28%). The racial/ethnic distribution was as follow: White (N=113), African American (N=15), Hispanic (N=4), Asian (N=2), and Other/Unknown (N=4) The ages of subjects ranged from 18 to 85 years. Not all women were able to complete all four data collection periods; hence, the subject number varies per data collection time period. Many women did not return for a follow-up clinic visit after biopsy. Also, some women chose not to complete some instruments for personal reasons. The psycho-endocrine stress profile and NK cell activity of each subject pre and post breast biopsy was measured. Each individual was administered psychological instruments, had blood drawn for stress hormone assessment and peripheral blood mononuclear cells were isolated to measure

NK cell activity and cytokine production. Relevant demographic, medical history, and disease characteristics from medical records were abstracted and contact with subjects was maintained per telephone to ensure subject retention, and quality control of laboratory and psychological data collection has been accomplished.

**Task 2.** Identify the importance of the Th1/Th2 cytokine profile to the psycho-endocrine-NK cell response of women undergoing breast biopsy for cancer diagnosis (month 1-48). For each of the blood samples from the above subject, cytokine production was measured pre and post biopsy.

**Task 3.** Understand differences in the psycho-endocrine-immune profile of women with benign versus malignant breast biopsy findings (months 24-48). Cytokine variables are presented for the all women and the stratification of this data into benign and malignant groups.

**Task 4.** Analysis and Manuscript Preparation is underway (months 24-48) one article has been published, one review chapter has been published, and one article is in preparation.

**Summary of Results:** This study investigated psychological indices of stress and immunological parameters. Results for all figures are reported in the same general manner and all Figures are located in the Appendix. The mean psychological and immunological results are indicated for pre biopsy time points (T1 and T2) and post biopsy time points (T3, and T4). The time frame for these measures is defined above in Methods. At each time point, results are presented for all women who underwent biopsy as well as for subgroups of these same women who ultimately had either benign or malignant findings. The biopsy groups are the following: **All:** All women undergoing biopsy no matter what the biopsy findings; **Benign:** women with benign biopsy findings, and **Malignant:** women with malignant biopsy findings. For each time point (T1-T4) comparisons were made within the biopsy groups. In addition, between group comparisons were made to compare each biopsy group to a group of non-biopsied control women.

**Psychological Assessments:** Figure 1 illustrates results for perceived stress (PSS) for women at pre biopsy (T1 and T2) and at post biopsy (T3 and T4), as well as perceived stress for control (non-biopsied) women. The results indicate that the period of biopsy was a time of reported perceived stress. All three groups of women at T2 and T3 reported perceived stress that was significantly greater than that of the non-biopsied control women. In addition, the All and Benign groups at T1 and T4 reported significantly greater perceived stress than control women. Of note, all three groups showed a numerical reduction in perceived stress post biopsy at T4.

In Figure 2, results are presented for the VAS (10-cm stress visual analogue scale), which indicates the overall stress in a woman's life at the moment. The results show that the two pre biopsy time points were times of psychological stress for all women. Global stress levels decreased at both post biopsy time points in all three groups of women. All three groups of women reported elevations in global stress at T2 and for the All and Benign groups at T1, when compared to non-biopsied, control women. No differences were observed between reported stress by biopsied women and non-biopsied, control women at T3 or T4.

Similar to the PSS, the POMS total mood disturbance score (POMS TMD) was elevated for women pre biopsy compared to control, non-biopsied women, Figure 3. Self-reported total mood disturbance was reduced pre to post biopsy, but the scores remained elevated when compared to

scores reported by control women. An exception was the scores reported at T4 by the women in the Malignant group, which did not differ from those of the control women. Similar to the PSS and the POMS-TMD were the scores reported for Anxiety. Anxiety was significantly elevated for women pre biopsy compared to control, non-stressed, women, Figure 4. Self-reported Anxiety was reduced pre to post biopsy, but the scores remained elevated when compared to scores reported by control women. An exception was the score reported at T4 by the women in the Malignant group, which did not differ from those of the control women.

The POMS is comprised of six subscales that measure Tension, Depression, Anger, Vigor, Fatigue, and Confusion. Figures 5-10 show changes over time for these mood subscales. In Figure 5 comparisons are presented to control (non-biopsied) women for the Tensions subscale of the POMS. The results indicate that the period prior to biopsy was a time of reported tension. All three groups of women at T2 and the All and the Benign groups at T1 reported tension that was significantly greater than the control women. Tension was reduced after biopsy. However, for the All and the Malignant groupings at T3 and the All and Benign groupings at T4, the tension was still significantly elevated compared to control women.

In Figure 6 comparisons are presented to control (non-stressed) women for the Depression subscale of the POMS. The results indicate that the period prior to biopsy was a time of reported depression. All three groupings of women at T2 and the All and the Benign groupings at T1 reported depression that was significantly greater than the non-stressed control women. This depression did not return to normal levels after biopsy, with the exception of the Malignant group at T4. The results of the POMS Anger subscale (POMS-A) indicate that the period prior to biopsy was a time of reported anger for some of the women (Figure 7). The All and Benign groupings at T1 reported anger that was significantly greater than that of the control women. Anger returned to control levels after biopsy.

As shown in Figure 8, the pre biopsy period was a time of reduced vigor (POMS-V). All three groups of women at T2 and T3 as well as the All and the Benign groups at T1 and T4 reported vigor that was significantly less than the control women. This decreased vigor did not return to normal levels after biopsy, with the exception of the Malignant group at T4. As for fatigue (POMS-F), the results are variable but indicate that for some women the period prior to biopsy was a time of increased fatigue (Figure 9). This was true for the All and Benign groups at T1 and for the All and Malignant groups at T2. After biopsy no differences in fatigue were noted compared to control women. Figure 10 illustrates that prior to biopsy women reported confusion (POMS-C). All three groups of women at T2 and the All and the Benign groups at T1 reported confusion that was significantly greater than the non-stressed, control women. Confusion did not return to normal levels after biopsy for the All group at T3 or for the Malignant grouping at T3.

**Immunological Assessments:** The NKCA for PBMC (lytic units at 20%) of women pre and post breast biopsy is illustrated in Figure 11. In comparison to the control women, NKCA was significantly depressed for both the All and Benign groups for each time period, with the exception of the Benign grouping at T4. Significant depression in NKCA was only observed after biopsy (T3 and T4) for the Malignant group. The NKCA of the Benign group at T4 was not statistically different from control women. Phenotypic analysis for circulating NK cells showed no differences between normal control subjects and biopsy patients at any time point (Figure 12).

Cytokine production by PBMC of women pre and post biopsy is shown in Figure 13 - 17. For IL-2

production, no significant changes were observed for any of the groups at any of the time periods, Figure 13. Those results are quite dissimilar to those obtained for IL-6, shown in Figure 14. For all biopsied groups, at all time points, the amount of produced IL-6 was significantly greater than the amount of IL-6 produced by control women. In contrast to those results, the capacity to produce interferon (IFN) gamma was significantly reduced for the All grouping at T2 and T3, and for the Benign grouping at T3, when the results were compared to control women (Figure 15). At all time points the quantity of produced IFN gamma was numerically less for the Malignant grouping than for any of the other two groupings. Much like IL-6, increased IL-4 and IL-10 production were observed in the biopsied women compared to the control women. In comparison to control women, IL-4 production was significantly increased for all groupings of biopsied women at T2 and for the Malignant grouping at T1 and T3 as well as for the All grouping at T3 (Figure 16). IL-4 production by all groups of women decreased from T2 to T4, and by T4, IL-4 production was not different from control women. IL-10 production by the PBMC of biopsied women was elevated in comparison to control women for all three groupings of biopsied women and for all time periods (Figure 17). No cytokines were detected in the serum of representative control or biopsy subjects. Data not shown.

**Endocrine Assessments** – In order to determine the stress hormone response to the stress of breast biopsy, plasma cortisol and DHEA-SO4 were measured. Psychological stress was hypothesized to lead to an increase in cortisol secretion and a decrease in DHEA-SO4 levels. It was also hypothesized that these hormonal changes would mediate a “switch” in TH1/TH2 cytokine production. As can be seen in Figures 18 and 19 no differences were observed in either cortisol or DHEA-SO4 over time (T1 through T4). However, cortisol at T2 is lower in the malignant group of women relative to benign and control groups; while DHEA-SO4 is also lower in the malignant women at T1 through T4.

**Modulation of the Psychological Response to Breast Cancer Diagnosis:** The role of enduring psychological constructs in the psychological response of women to the experience of breast cancer diagnosis was determined. Two constructs were evaluated in our group of study subjects: Sense of Coherence (SOC) and Resilience (RSL). SOC and RSL were postulated to modulate coping and adaptational responses to psychological and physiological stress, including immune dysfunction, and thus may provide important clues for the identification of women at risk for experiencing high levels of psychological stress during breast biopsy, regardless of results. In addition, SOC and RSL may have predictive values regarding psychological stress and adaptation to cancer in women with malignant results during the experience of breast biopsy. No study has been identified that has evaluated RSL and SOC with regard to effect upon psychological stress in women undergoing the stressful event of breast biopsy. Hence, SOC and RSL were evaluated in women undergoing diagnostic breast biopsy.

Tables 1 and 2 show the relationships among SOC, RSL and perceived stress (PSS) in women with benign breast biopsy results and in women with malignant breast biopsy results, respectively. In both groups of women, benign and malignant, there was a significant positive relationship between SOC and RSL; which was more marked in the malignant group. SOC was negatively associated ( $p < 0.02$ ) with perceived stress (PSS), total mood disturbance (TMD), and state anxiety (STAI) in both the groups of women, benign and malignant. Similarly, a significant negative relationship was observed between RSL and PSS and between RSL and TMD in both the benign and malignant groups. However, only the women in the malignant group exhibited a

significant negative relationship between RSL and STAI. Interestingly, all relationships were much stronger in the group of women diagnosed with breast cancer compared to the group of women with benign breast biopsy results.

These findings suggest that SOC and RSL may buffer the psychological response to stress and provide preliminary data for the development of an explanatory model, which can guide psychological risk assessment in women undergoing cancer diagnosis. Early identification of psychological risk can target interventions to facilitate adaptation. Future analysis will explore the role of SOC and RSL in adaptation to cancer and quality of life in breast cancer survivors.

### **(3) KEY RESEARCH ACCOMPLISHMENTS:**

- A psycho-endocrine-immune assessment of women at two time periods pre and two time periods post breast biopsy has been accomplished.
- Stress, perceived stress, anxiety, and mood disturbance are heightened in women pre biopsy and in some cases began to "normalize" post biopsy.
- Perceived stress, anxiety, and mood disturbance diminish post biopsy but remain elevated in comparison to non-biopsied, control women.
- Despite the psychological stress changes observed in women undergoing breast biopsy, no differences in the stress hormones, cortisol and DHEA-SO4 were observed from T1 through T4.
- Women in both benign and malignant groups exhibited significant inverse relationships between sense of coherence and perceived stress and mood disturbance; also, resilience was inversely related to perceived stress and mood disturbance.
- NK cell activity is depressed in women both pre and post breast biopsy compared to non-biopsied control women.
- Cytokine dysregulation accompanies the depression in NK cell activity.

### **(4) REPORTABLE OUTCOMES:**

#### **Bibliography of Publications and Meeting Abstracts:**

Witek-Janusek, L. and Mathews, H.L. Stress, Immunity, and Health. 2000. In: *Handbook of Stress and Coping*, V. Rice (Ed.), Sage Publications Inc., Thousand Oaks.

Nagabusham, M., Mathews, H.L., and Witek-Janusek, L. 2001. Aberrant nuclear expression of AP-1 and NFkB in lymphocytes of women stressed by the experience of breast biopsy. *Brain, Behavior, and Immunity*. 15: 78-84.

Robinson, F.P., Mathews, H.L., and Witek-Janusek, L. (2002). Issues in the design and implementation of psychoneuroimmunology research. *Biological Research for Nursing*, 3:163-175.

Witek-Janusek, L. and H. L. Mathews. Immunological and Psychological Response of Women Experiencing Breast Biopsy, in preparation, *Brain Behav. Immun*, 2002.

### **Meeting Abstracts:**

TH1/TH2 cytokine production during the stress of breast cancer diagnosis (1999). *Cytokine* 11:951. Presented at the Seventh Annual Conference of the International Cytokine Society, Hilton Head, South Carolina, December, 1999.

Herbert L. Mathews, Moolky Nagabhushan, and Linda Witek-Janusek. Aberrant nuclear expression of AP-1 and NFkB in lymphocytes of women stressed by the experience of breast biopsy. *Brain Behavior and Immunity* 14: 114, 2000. Presented at Cape Fear-Wilmington, North Carolina, Annual Meeting of the Psychoneuroimmunology Research Society Meeting, May, 2000.

Witek-Janusek, L. and Mathews, H.L. (2000). Cytokine production and natural killer cell activity in women stressed by breast cancer diagnosis. *Brain, Behavior, and Immunity*, 14: 139. Presented at Cape Fear-Wilmington, North Carolina, Annual Meeting of the Psychoneuroimmunology Research Society Meeting, May, 2000.

Psycho-endocrine-immune profile: Implication for quality of life in breast cancer patients. Presented at *Era of Hope*, Department of Defense Breast Cancer Research Program Meeting, Atlanta, GA, June, 2000.

Witek-Janusek, L. and Sorenson, M. "Bridging the Gap Between Mind and Body: Psychoneuroimmunology Research," Ruth K. Palmer Annual Research Symposium, Loyola University of Chicago, March, 2002.

Keating, P. Witek-Janusek, L. and Mathews, HL. "Modulation of the Psychological Response to Breast Cancer Diagnosis," Ruth K. Palmer Annual Research Symposium, Loyola University of Chicago, March, 2002.

Mathews, H.L. and Witek-Janusek, L. (2002). The psychological impact of breast cancer diagnosis produces prolonged alterations in TH1/TH2 cytokines and natural killer cell activity. *Brain, Behavior, and Immunity*, 16: 179. Presented at the Annual Psychoneuroimmunology Research Society Meeting, Madison, Wisconsin.

### **Degrees:**

Pam Keating – Ph.D. student, Loyola University of Chicago, School of Nursing; Ms. Keating has worked on this DOD-funded project as a research assistant and will extend the work related to modulation of the psychological response to breast cancer diagnosis by sense of coherence and resilience. This will form the foundation for her dissertation research. She has written her preliminary examination paper and will defend it orally in December, 2002. The paper is titled: "Psychological Stress and Sense of Coherence: Bridging the Gap."

**Funding Applied For:**

Agency: Department of Defense Breast Cancer Research Fund

Title: "Mindfulness: A Psychological and Immunological Intervention for Breast Cancer Survivors"

Submitted: June 2001

Not Funded

Agency: National Institutes of Health, National Cancer Institute

Title: "MBSR for Stress-Related Immune Dysregulation in Cancer"

Submitted: February 2002

Status: Pending (Score 179)

Agency: National Institutes of Health, National Institute for Nursing Research

Title: "Stress, Immunity, and Decision Making in DCIS"

Submitted: June 2002

Status: Pending

**Personnel Receiving pay from this research effort:**

Pamela J. Keating

Maribel Barrigan

Karlee Koning

Deanne Szymanski

Jonna Peterson

**(4) CONCLUSIONS:**

In order to determine whether psychological stress accompanies breast biopsy, scores on various stress measures pre and post biopsy were compared to non-biopsied control women. The results clearly showed that women undergoing breast biopsy scored higher than did age-matched, non-biopsied, control women for all of the psychological measures evaluated. Moreover, pre biopsy subjects reported significantly more psychological stress, more perceived stress, experienced more anxiety, and more total mood disturbance, than did the subjects post biopsy. In conclusion, these results demonstrate the experience of breast biopsy to be a psychologically stressful event. It is also noteworthy that, with the exception of the VAS, emotional distress and mood disturbance remained elevated after biopsy. For the majority of women, the POMS-TMD and the Anxiety index (STAI) showed reduction pre biopsy to post biopsy, but this was not the case for the PSS, which remained similar to pre biopsy scores. However, for most of the POMS subscales, values pre biopsy were still greater than those reported by control women. A reduction in scores was reported for tension, depression, and confusion pre to post biopsy. In the case of vigor, pre biopsy women reported less vigor than post biopsy women did and post biopsy women reported less vigor than non-biopsied, control women did. These data clearly show that the experience of breast biopsy produced psychological distress and mood disturbance in women that extended well beyond the day of biopsy. In particular, increased tension and depression and reduced vigor significantly contributed to the mood disturbance after biopsy with minor contribution by confusion. In addition, women with greater sense of coherence and resilience reported less perceived stress and total mood disturbance; these relationships were much stronger in the group of women diagnosed with breast cancer.

Assessment of these personal resources (sense of coherence and/or resilience) may assist in identifying women at risk for negative psychological sequelae during the experience of breast cancer diagnosis.

Th1, Th2, and proinflammatory cytokine production fell into three distinct categories for the biopsy subjects. As a group, production of cytokines by the PBMCs of the biopsied women either remained unchanged (IL-2), showed an increase compared to control subjects (IL-4, IL-6, and IL-10), or alternatively showed a reduction when compared to control subjects (IFN gamma). IL-2 has been characterized as a Th1 cytokine and falls into the first category. IL-2 production by the majority of biopsy patients (as a whole group) was unchanged from pre to post biopsy and was approximately the same as that of control subjects. IL-6 is a pro-inflammatory cytokine that falls into the second category. IL-6 production by all biopsy patients was greater than that observed in control subjects with increased levels of production, pre and post biopsy. In this category as well are IL-4 and IL-10, which are both Th2 cytokines that showed markedly increased production during the biopsy experience. IFN gamma, a Th1 cytokine, falls into the third category and showed decreased production by the majority of biopsy patients in comparison to control subjects. In addition to these measures, a marked reduction was observed in NKCA between control, non-biopsied, women and women who experienced biopsy. Women entered into the study were aware that breast biopsy was impending and as described above, experienced psychological stress pre biopsy. The impact upon NKCA appeared early (T1) and continued throughout the post biopsy period. The pre biopsy time points showed a significant diminution in mean NKCA for the majority of women. Because the reduction in NKCA was not accompanied by a change in the number of circulating NK cells these data clearly suggest that the observed reduction in NKCA is a consequence of reduced NKCA and not a reduction in NK cell number. As noted above significant emotional distress and mood disturbance was observed in women post biopsy when compared to that of control women. Collectively, these results suggest that this distress appears to have, in turn, produced long-term effects on immune function; given that reduced NKCA and dysregulation in cytokine production continued beyond the day of biopsy.

In conclusion, these results provide evidence that the stress of breast biopsy for cancer diagnosis, leads to prolonged periods of stress, anxiety, and mood disturbance that appear to be associated with depressed NKCA and an altered pattern of cytokine production. The extent of stress and mood disturbance is related to a woman's sense of coherence and resilience; these constructs may buffer the stress of cancer diagnosis. Importantly, it appears that stress-induced alterations in the immune system are not transient but persist beyond the acute experience of breast biopsy. This may be of particular relevance to women diagnosed with malignancy since they face additional stressors related to cancer treatment and adaptation to illness. Suppression of NKCA may be of relevance to cancer control since NK cells contribute to protection from tumors (13, 19, 28, 30) including tumor initiation (29, 33), primary tumor growth (33), and tumor metastasis (29, 33).

**Evaluation of Knowledge as a Scientific/Medical Product** – These results provide evidence that the stress of breast cancer diagnosis leads to prolonged periods of perceived stress, anxiety, and mood disturbance, which appear to be associated with depressed NK cell activity and an altered pattern of cytokine production. Stress-induced alterations in immunity are not transient but persist beyond the acute experience of the biopsy. This may be of particular relevance to women diagnosed with malignancy since they will be facing additional stressors

related to cancer treatment and adaptation to illness. This data set supports the need to incorporate stress-reduction strategies and to provide emotional support to women during the earliest stages of the cancer trajectory. Multi-disciplinary approaches to cancer diagnosis and treatment should seriously consider the incorporation of such approaches in the holistic care of women facing a cancer diagnosis. As this data supports, such approaches might prove to be beneficial to both the psychological and immunological status of women with cancer.

Women who are psychologically disturbed by the experience of breast biopsy have a decrease in NK cell tumoricidal activity compared to women who are not undergoing the experience of breast biopsy. Since NK cells mediate natural resistance against tumor cells and are particularly important in the control of tumor metastasis, the observed reductions in NK cell activity have implications for the control of cancer progression and hence the quality of life of women with breast cancer. Important regulators of NKCA are cytokines that are produced by subsets of lymphocytes and the relative balance of these cytokines can impact NK cell activity. Th1 lymphocytes produce IL-2 and IFN- $\gamma$ . NKCA is promoted by these cytokines released by Th1 lymphocytes. Th2 lymphocytes produce IL-4, and IL-10. Th2 cells strongly support B lymphocyte activation and immunoglobulin production but contribute little to NK cell activity. These results indicate a diminution in the overall production of IFN- $\gamma$ , with concomitant increases in IL-4, IL-6, and IL-10.

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## Appendix

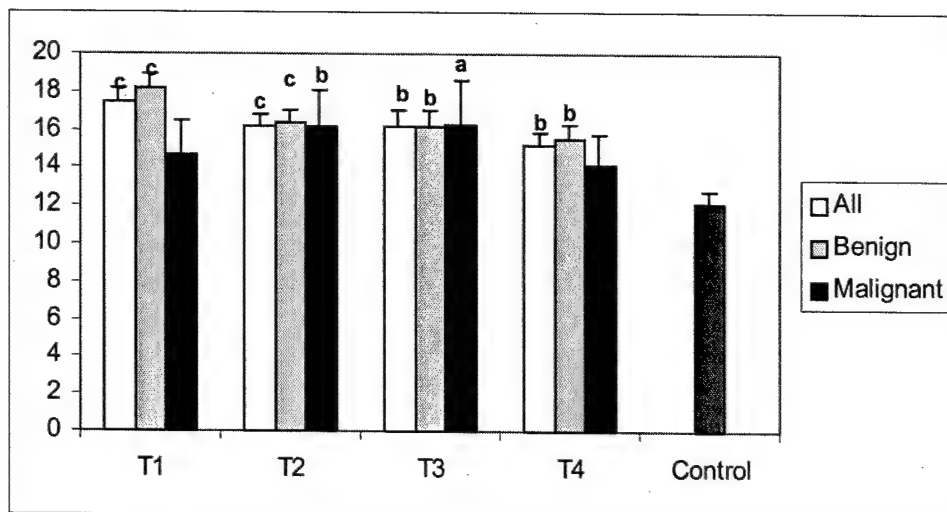


Figure 1. Psychological measure of perceived stress is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Perceived stress was measured using Cohen's Perceived Stressor Scale (PSS). N for; All at T1= 85, All at T2= 108, All at T3= 86, All at T4= 87, Control=49. Benign at T1= 68, Benign at T2= 85, Benign at T3= 66, Benign at T4= 67. Malignant at T1= 18. Malignant at T2= 22, Malignant at T3= 22, Malignant at T4= 19. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

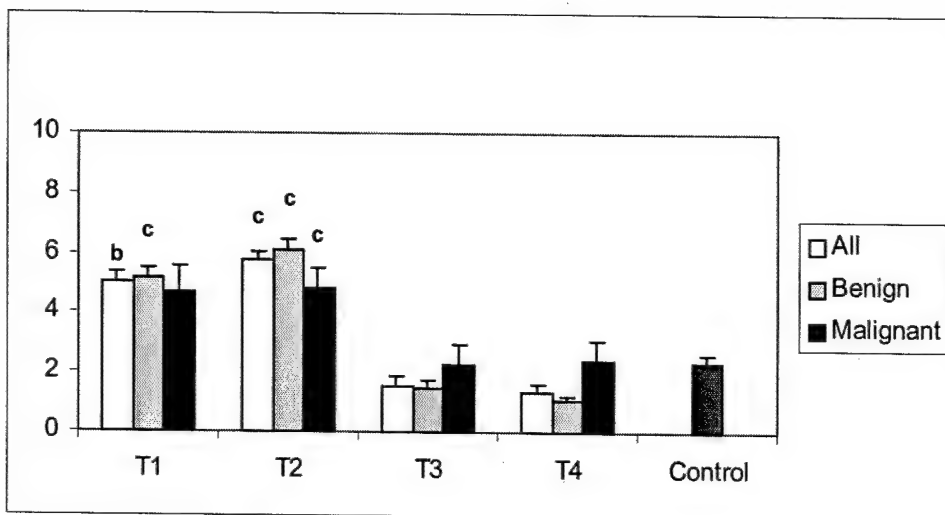


Figure 2. Psychological measure of stress is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Stress was measured by use of 10 cm visual analogue scales that determined global stress (VAS). N for; All at T1= 87, All at T2= 106, All at T3= 63, All at T4= 68, Control=45. Benign at T1= 69, Benign at T2= 80, Benign at T3= 50, Benign at T4= 52. Malignant at T1= 19, Malignant at T2= 25, Malignant at T3= 15, Malignant at T4= 15. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

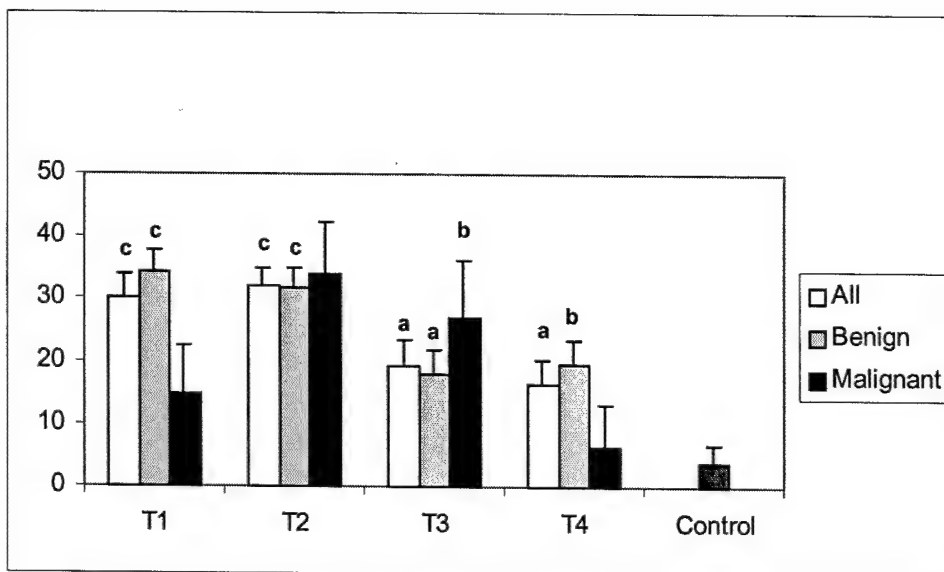


Figure 3. Psychological measure of mood state is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Mood state was measured using the Profile of Mood States (POMS) and the total mood disturbance (TMD) is depicted. N for; All at T1= 81, All at T2= 106, All at T3= 88, All at T4= 88, Control=50. Benign at T1= 64, Benign at T2= 83, Benign at T3= 67, Benign at T4= 68. Malignant at T1= 18, Malignant at T2= 22, Malignant at T3= 23, Malignant at T4= 19. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

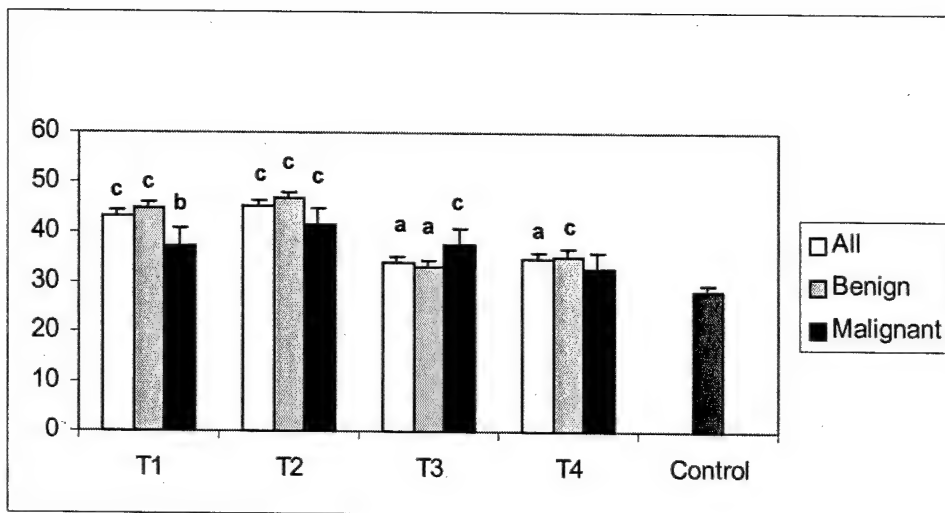


Figure 4. Psychological measure of anxiety is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Anxiety was measured using Spielberger's State Anxiety Inventory (STAI). N for; All at T1= 86, All at T2= 111, All at T3= 88, All at T4= 89, Control=29. Benign at T1= 69, Benign at T2= 84, Benign at T3= 68, Benign at T4= 68. Malignant at T1= 18, Malignant at T2= 26, Malignant at T3= 22, Malignant at T4= 20. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

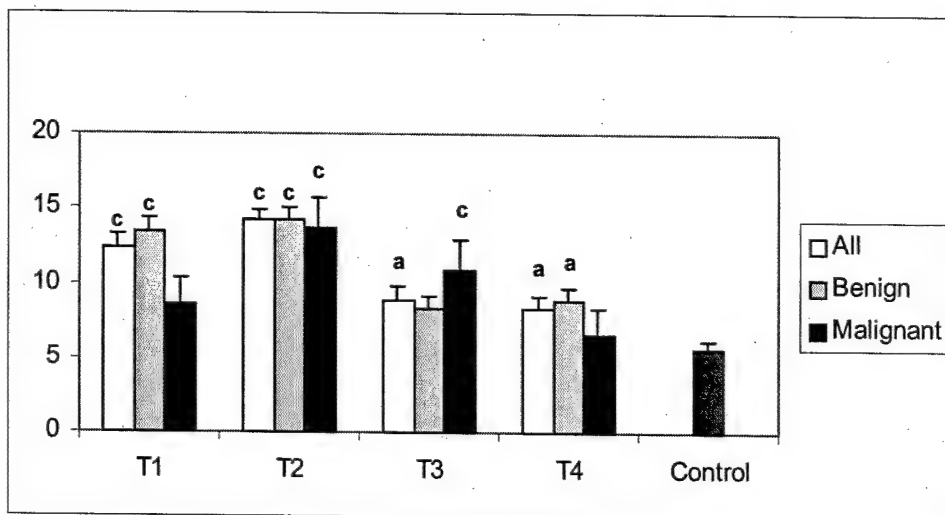


Figure 5. The tension subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 81, All at T2= 108, All at T3= 88, All at T4= 89, Control=50. Benign at T1= 65, Benign at T2= 84, Benign at T3= 67, Benign at T4= 69. Malignant at T1= 17, Malignant at T2= 22, Malignant at T3= 23, Malignant at T4= 19. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ . Statistical comparison T1 to T2 for biopsied women; d= $p \leq 0.05$ , e= $p \leq 0.01$ , f= $p \leq 0.001$ .

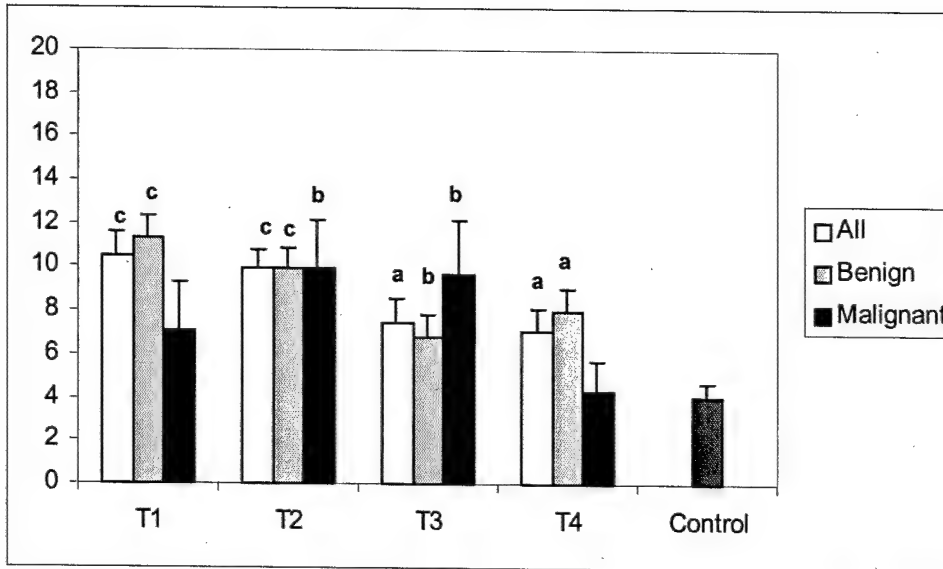


Figure 6. The depression subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 80, All at T2= 106, All at T3= 88, All at T4= 88, Control=50. Benign at T1= 64, Benign at T2= 83, Benign at T3= 67, Benign at T4= 68. Malignant at T1= 17, Malignant at T2= 22, Malignant at T3= 23, Malignant at T4= 19. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

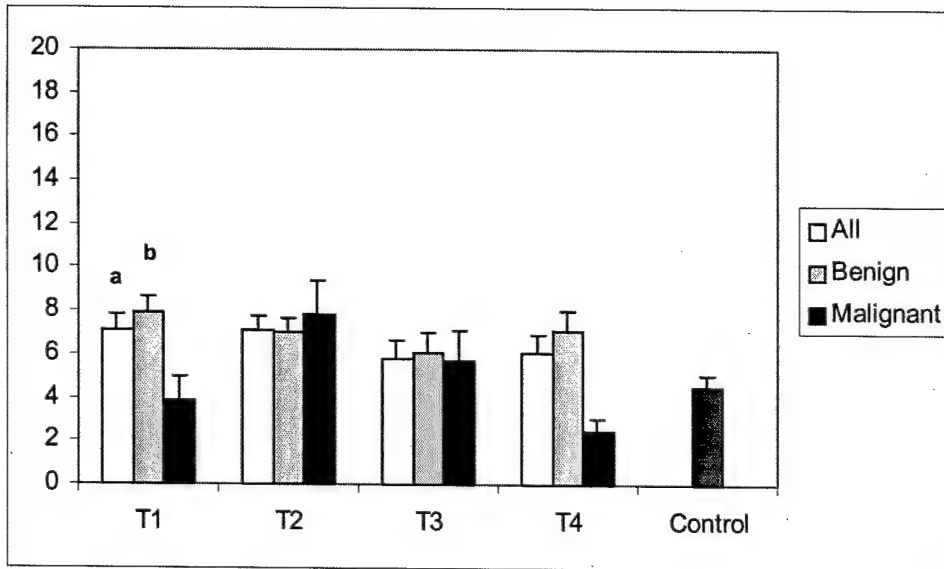


Figure 7. The anger subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 81, All at T2= 105, All at T3= 88, All at T4= 89, Control=50. Benign at T1= 64, Benign at T2= 82, Benign at T3= 68, Benign at T4= 69. Malignant at T1= 18, Malignant at T2= 22, Malignant at T3= 21, Malignant at T4= 19. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

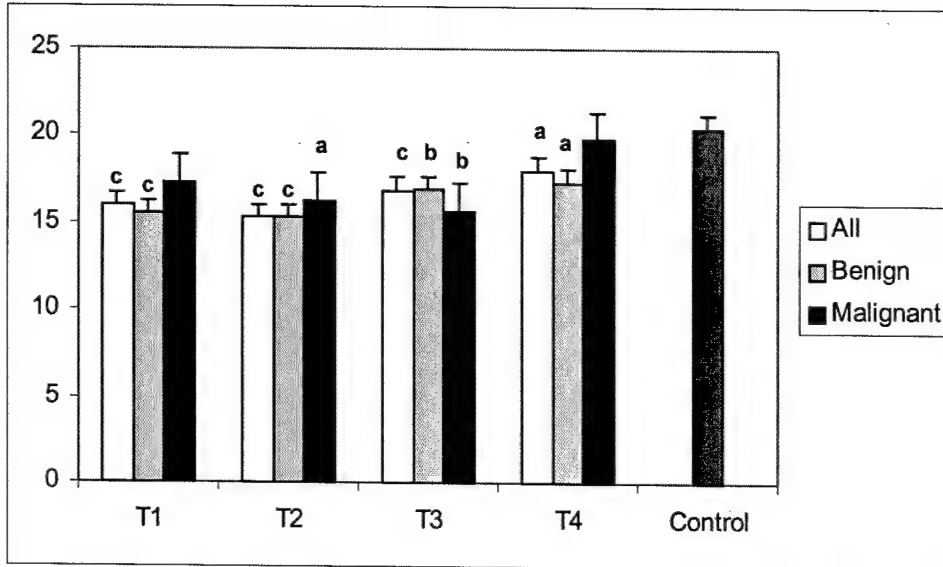


Figure 8. The vigor subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 80, All at T2= 106, All at T3= 88, All at T4= 88, Control=50. Benign at T1= 64, Benign at T2= 83, Benign at T3= 67, Benign at T4= 68. Malignant at T1= 17, Malignant at T2= 22, Malignant at T3= 23, Malignant at T4= 19. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

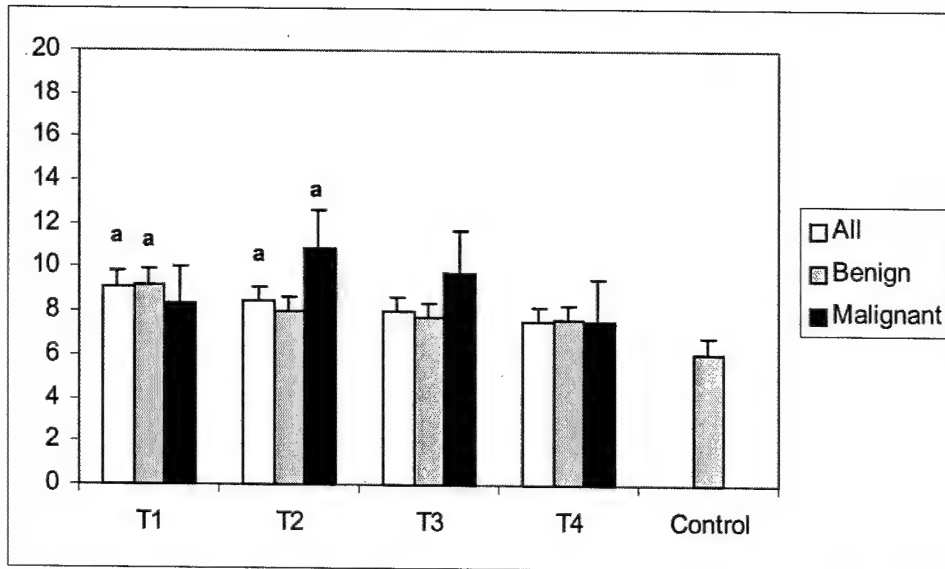


Figure 9. The fatigue subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 80, All at T2= 106, All at T3= 88, All at T4= 88, Control=50. Benign at T1= 64, Benign at T2= 82, Benign at T3= 67, Benign at T4= 68. Malignant at T1= 17, Malignant at T2= 22, Malignant at T3= 23, Malignant at T4= 19. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

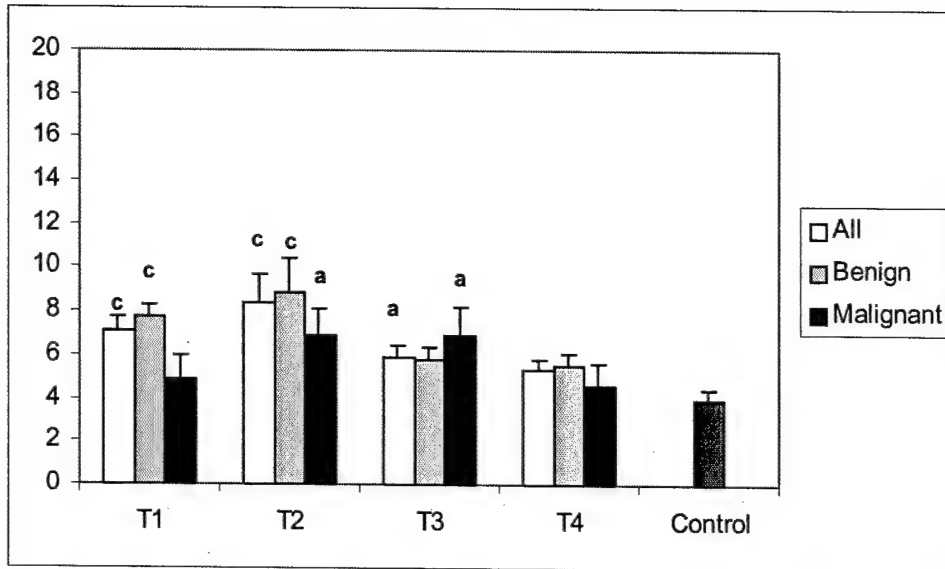


Figure 10. The confusion subscale of the POMS is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. N for; All at T1= 81, All at T2= 106, All at T3= 88, All at T4= 88, Control=50. Benign at T1= 64, Benign at T2= 83, Benign at T3= 67, Benign at T4= 68. Malignant at T1= 18, Malignant at T2= 22, Malignant at T3= 23, Malignant at T4= 19. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq .01$ , c= $p \leq 0.001$ .

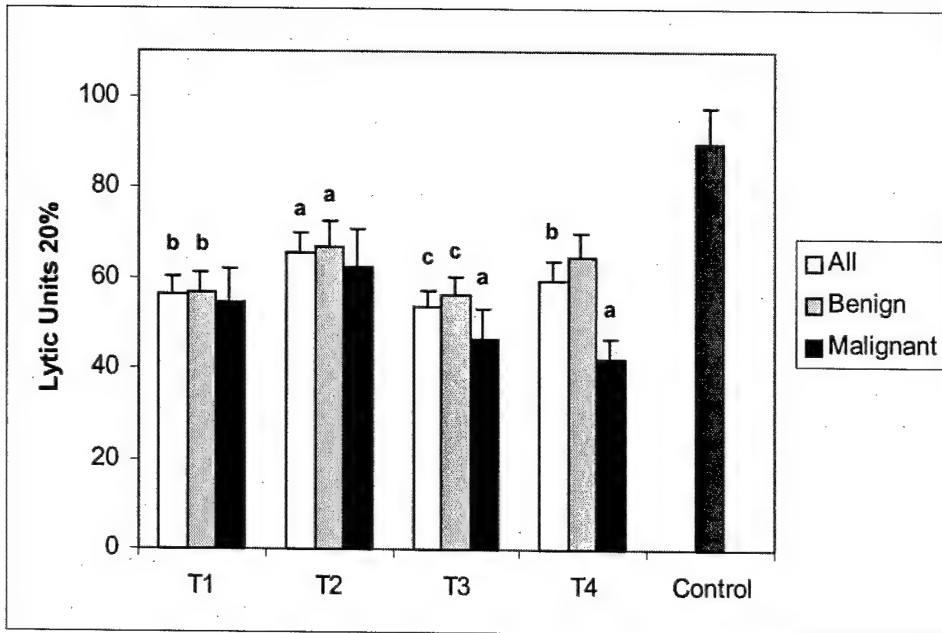


Figure 11. NKCA, expressed as lytic units at 20%, is illustrated for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. NKCA was measured using K562 tumor cells as the target. N for; All at T1= 85, All at T2= 126, All at T3= 98, All at T4= 84, Control=20. Benign at T1= 68, Benign at T2= 94, Benign at T3= 74, Benign at T4= 63. Malignant at T1= 17, Malignant at T2= 30, Malignant at T3= 24, Malignant at T4= 23. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

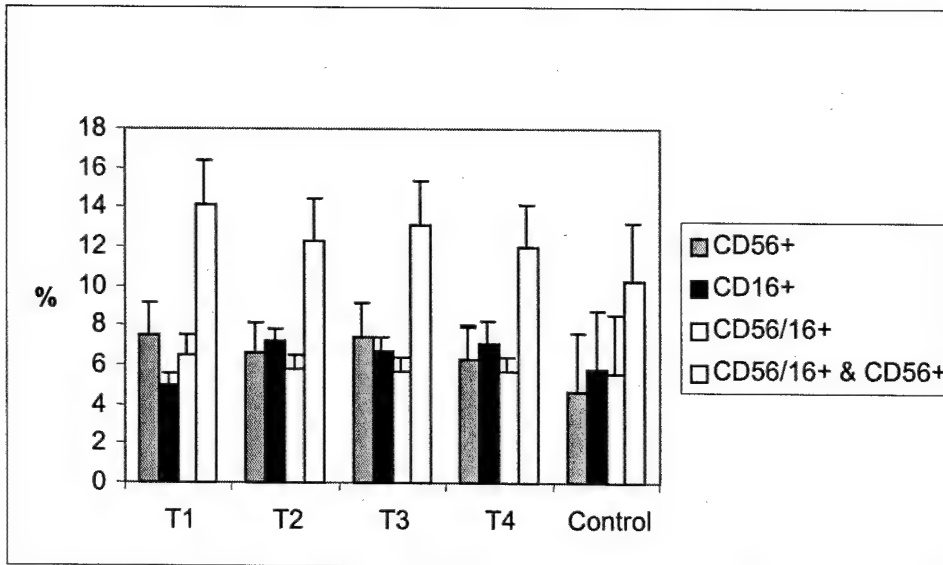


Figure 12. Phenotypic analysis of PBMC for all biopsied women. N for; T1 CD56+ = 28, CD16+ = 28, CD56/16+ = 28, CD56/16 & CD56+ = 28, T2 CD56+ = 44, CD16+ = 44, CD 56/16+ = 44, CD56/16 & CD56+ = 44, T3 CD56+ = 31, CD16+ = 31, CD56/16+ = 31, CD56/16 & CD56+ = 31, T4 CD56+ = 23, CD16+ = 23, CD56/16+ = 23, CD56/16+ & CD56+ = 23, T1 Control CD56+ = 12, T2 Control CD16+ = 12, T3 Control CD 56/16+ = 12, T4 Control CD56/16+ & CD56+ = 12.

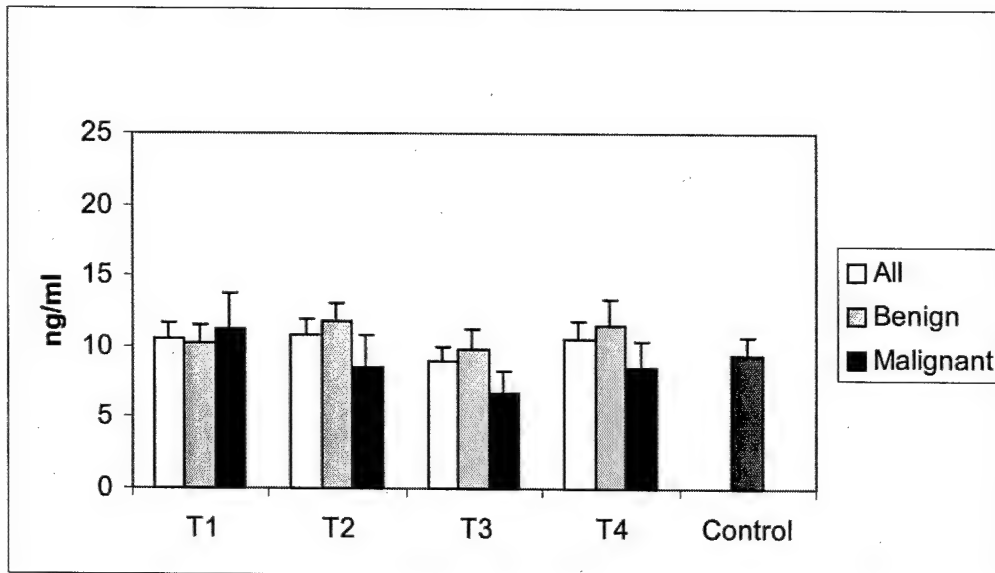


Figure 13. PBMC production of IL-2 is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; All at T1= 66, All at T2= 87, All at T3= 69, All at T4= 61, Control=29. Benign at T1= 48, Benign at T2= 61, Benign at T3= 50, Benign at T4= 40. Malignant at T1= 18, Malignant at T2= 24, Malignant at T3= 18, Malignant at T4= 21. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

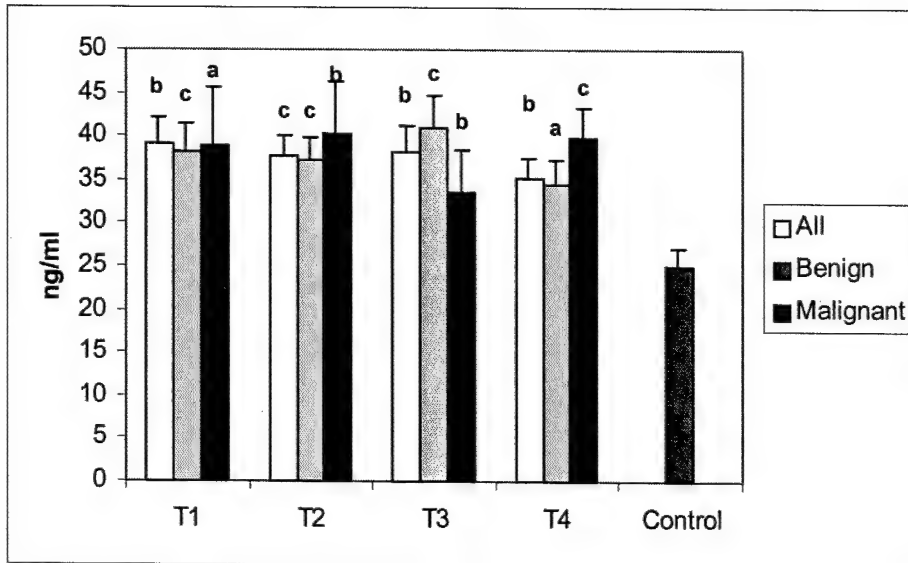


Figure 14. PBMC production of IL-6 is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; All at T1= 75, All at T2= 106, All at T3= 91, All at T4= 80, Control=37. Benign at T1= 57, Benign at T2= 75, Benign at T3= 66, Benign at T4= 59. Malignant at T1= 15, Malignant at T2= 22, Malignant at T3= 21, Malignant at T4= 19. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

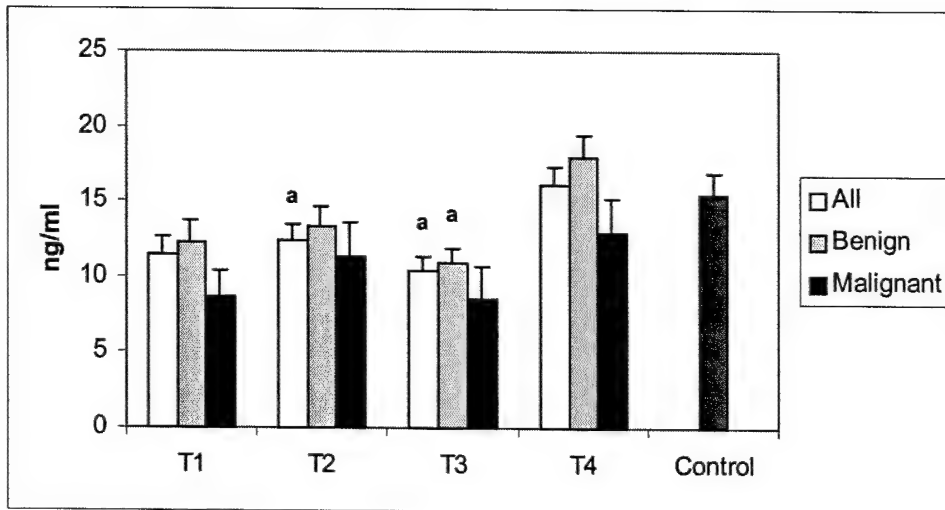


Figure 15. PBMC production of IFN  $\gamma$  is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; All at T1= 71, All at T2= 99, All at T3= 81, All at T4= 72, Control=32 Benign at T1= 55, Benign at T2= 71, Benign at T3= 59, Benign at T4= 48. Malignant at T1= 16, Malignant at T2= 27, Malignant at T3= 23, Malignant at T4= 22. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

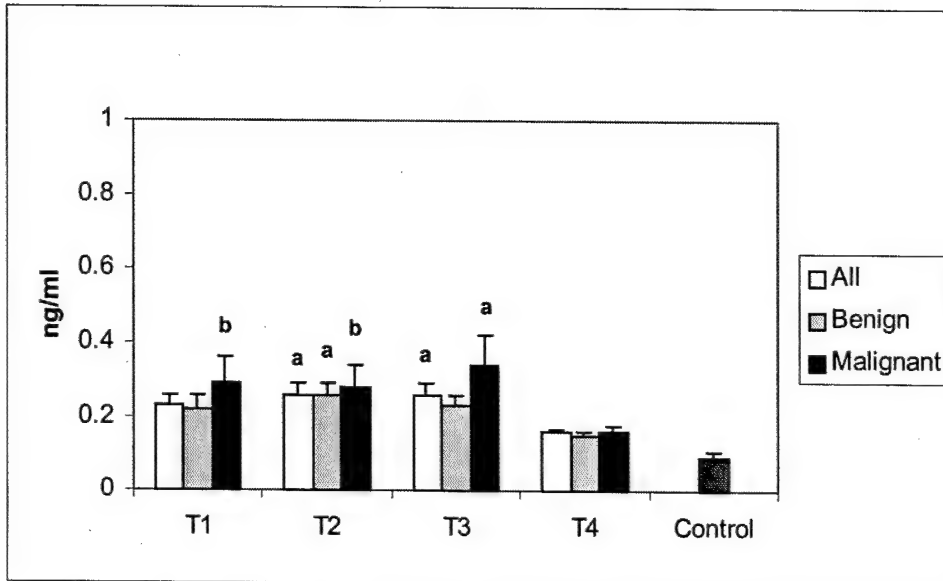


Figure 16. PBMC production of IL-4 is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; All at T1= 77, All at T2= 107, All at T3= 92, All at T4= 84, Control=29. Benign at T1= 60, Benign at T2= 82, Benign at T3= 70, Benign at T4= 59. Malignant at T1= 17, Malignant at T2= 23, Malignant at T3= 24, Malignant at T4= 23. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

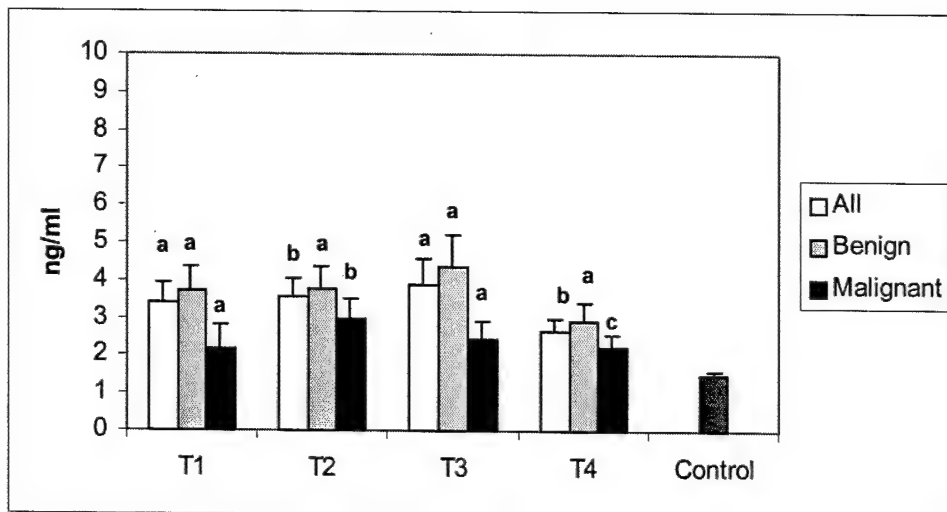


Figure 17. PBMC production of IL-10 is depicted for all biopsied women as well as for those women with benign findings, malignant findings, and in comparison to non-biopsied control women. Peripheral blood was collected pre and post breast biopsy and from control women. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. N for; All at T1= 78, All at T2= 112, All at T3= 89, All at T4= 84, Control=38. Benign at T1= 62, Benign at T2= 82, Benign at T3= 67, Benign at T4= 61. Malignant at T1= 16, Malignant at T2= 27, Malignant at T3= 22, Malignant at T4= 22. Bars represent the mean values  $\pm$  S.E. Statistical comparisons for all figures are as follows. Statistical comparison between experimental mean and control: a= $p \leq 0.05$ , b= $p \leq 0.01$ , c= $p \leq 0.001$ .

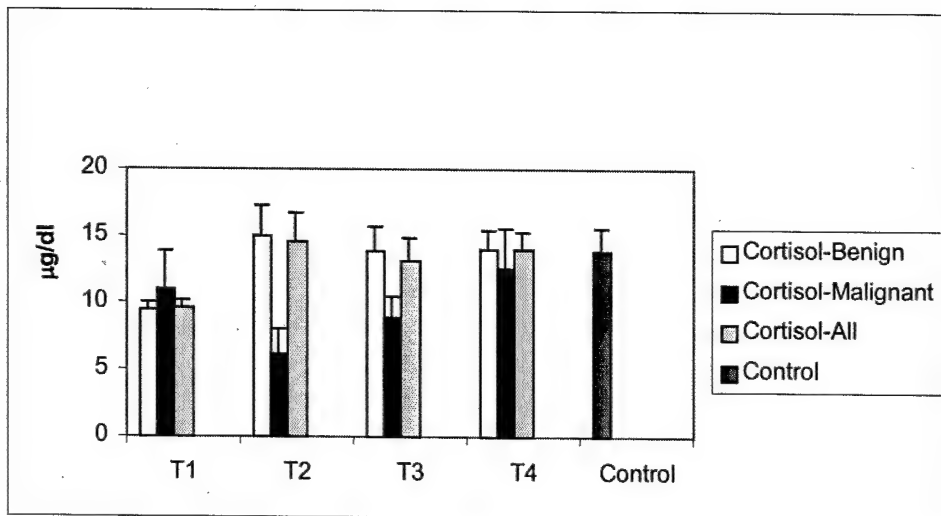


Figure 18. Cortisol concentrations depicted for all biopsied women and for women with benign findings, malignant findings, and in comparison to non-biopsied control women. Cortisol was measured by radioimmune assay. Cortisol: N for; Benign at T1=61 , Benign at T2=69 , Benign at T3=62 , Benign at T4=62 . Malignant at T1=7 , Malignant at T2=10 , Malignant at T3=10 , Malignant at T4=8 . Control=15. All at T1=68, All at T2=79, All at T3=72, All at T4=70. Bars represent the mean values  $\pm$  S.E.

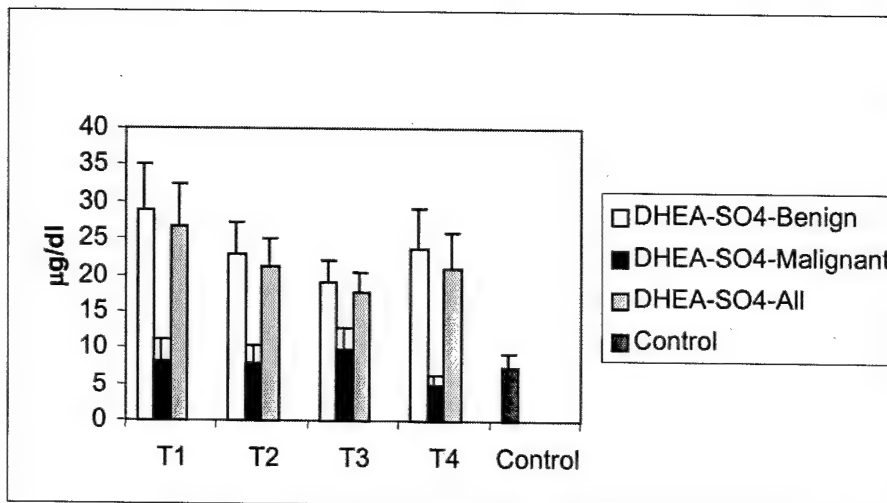


Figure 19. DHEASO4 concentrations depicted for all biopsied women and for women with benign findings, malignant findings, and in comparison to non-biopsied control women. DHEASO4 was measured by radioimmune assay. DHEASO4: N for; Benign at T1=60 , Benign at T2=69 , Benign at T3=59 , Benign at T4=57. Malignant at T1=7, Malignant at T2=10, Malignant at T3=10, Malignant at T4=10. Control=14. All at T1=67, All at T2=79, All at T3=69, All at T4=67. Bars represent the mean values  $\pm$  S.E.

**Table 1. Sense of Coherence, Resilience, and Stress in Women With Benign Biopsy Results**

<u>Variables</u>	<u>Correlation</u>	<u>Significance</u>	<u>N</u>
SOC and RSL	.587	.000	71
SOC and PSS	-.638	.000	52
SOC and TMD	-.606	.000	51
SOC and STAI	-.357	.010	51
RSL and PSS	-.424	.002	51
RSL and TMD	-.424	.002	50
RSL and STAI	-.238	.095	50

**SOC** = Sense of Coherence

**RSL** = Resilience

**PSS** = Perceived Stress

**STAI** = State Anxiety Inventory

**TMD** = Profile of Mood States - Total Mood Disturbance

**Table 2. Sense of Coherence, Resilience, and Stress in Women With Breast Cancer**

<u>Variables</u>	<u>Correlation</u>	<u>Significance</u>	<u>N</u>
SOC and RSL	.743	.000	27
SOC and PSS	-.771	.000	17
SOC and TMD	-.736	.001	16
SOC and STAI	-.567	.021	16
RSL and PSS	-.685	.002	17
RSL and TMD	-.841	.000	16
RSL and STAI	-.755	.001	16

**SOC** = Sense of Coherence

**RSL** = Resilience

**PSS** = Perceived Stress

**STAI** = State Anxiety Inventory

**TMD** = Profile of Mood States - Total Mood Disturbance

## Aberrant Nuclear Expression of AP-1 and NFkB in Lymphocytes of Women Stressed by the Experience of Breast Biopsy

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We have investigated the expression of AP-1 and NFkB in peripheral blood lymphocytes of women scheduled for breast biopsy. Samples were collected when women were informed of the need for biopsy (prebiopsy, T1, 5–7 days prior to the actual biopsy) and 7–10 days after they learned the result of their biopsy (postbiopsy, T2). At the time of blood collection, psychological stress was evaluated using Spielberger's State Trait Anxiety Inventory (STAI) and the Profile of Mood States (POMS). Women scheduled to undergo breast biopsy reported significant increases in anxiety (STAI) and mood disturbance (POMS). Gel shift mobility assays showed that mitogen stimulated peripheral blood lymphocytes of these women were less capable of the nuclear expression of AP-1 or NFkB at T1. Similar assessments, 7–10 days after the women learned of the results of their breast biopsy, showed these same women to have a marked reduction in anxiety and mood disturbance and an increased nuclear translocation of AP-1 and NFkB. These results show a significant decrease in nuclear AP-1 and NFkB expression during the period of emotional distress prior to biopsy with a return of nuclear transcription activity to normal levels when distress was relieved. Several studies have correlated increased psychological stress with decreased immune function. The results of this study suggest that psychological stress may mediate immunosuppression by altering the expression of the transcription factors, AP-1 and NFkB.

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**Key Words:** transcription factors; NFkB; AP-1; breast biopsy; psychological stress; human peripheral blood lymphocytes; gel shift assay; cancer.

### INTRODUCTION

A number of studies have explored the relationship between psychological stress and the immune response (Glaser, Rice, Sheridan, Fertel, Stout, Speicher, Pinsky, Kotur, Post, Beck, & Kiecolt-Glaser, 1987; Whiteside & Herberman, 1994). Those studies have assessed stress and immunity in medical students during classroom examinations, caregivers of diseased patients, individuals undergoing exercise-induced stress, volunteers exposed to experimental laboratory stress, and women with breast cancer (Glaser, Rice, Sheridan, Fertel, Stout, Speicher, Pinsky, Kotur, Post, Beck, & Kiecolt-Glaser, 1987; Whiteside & Herberman, 1994; Anderson, Farrar, Golden-Kreutz, Kreutz, Kutz, Maccallum, Courtney, & Glaser, 1998). Stressed individuals showed decreased lymphoblast transformation in response to mitogens (Dobbin, Harth, McCain, Martin, & Cousin, 1991); increased Epstein-Barr virus, Herpes simplex virus, and cytomegalovirus reactivation (Bonneau, Zimmerman, Ikeda, & Jones, 1998); dysregulation of cytokines (Marshall Jr, Agarwal, Loyod, Cohen, Henninger, & Morris, 1998); elevated secretion of proinflammatory cytokines; and decreased natural killer cell activity (Mae, Song, Lina, Jongh, Van Gastel, Kenis, Bosmans, De Meester, Benoy, Neels, Demedts, Janca, Scharpe, & Smith, 1998; Whiteside & Herberman, 1994).



NFkB and AP-1 are transcription factors that regulate lymphocyte function. Nuclear expression of these transcription factors is increased during lymphocyte activation, cytokine secretion, and latent viral reactivation. Agents that are known to block lymphocyte activation are immunosuppressive and inhibit NFkB and AP-1 activity (Kopp & Ghosh, 1995). Thus we hypothesized that stress may alter the expression of these transcription factors. Experiments were designed to evaluate the expression of NFkB and AP-1 in the peripheral blood lymphocytes isolated from women pre- and postbreast biopsy. This experimental design provides a naturalistic psychological stress paradigm in which the effects of stress can be evaluated.

### METHODS

Mononuclear cells were prepared from heparinized whole blood by Ficoll-Hypaque separation (Sigma, St Louis, MO). Mononuclear cells were cultured at a density of  $1 \times 10^6$ /ml/well in triplicate with or without phorbol 12-myristate 13-acetate and phytohemagglutinin (PMA + PHA), for 48 h at 37°C as described previously (Witek-Janusek & Mathews, 1999). Phenotypic analysis of mononuclear cells was performed with fluochrome conjugated antisera by flow cytometry with a Becton-Dickinson FACStar Plus System (Hialeah, FL). T lymphocytes were identified with anti-CD3 $\epsilon$ , B lymphocytes with anti-Ig, and NK cells with anti-CD56, from Pharmingen (San Diego, CA). The nonadherent cells (>99% lymphocytes as judged by Wright-Giemsa staining) were then harvested, treated with lysing buffer (10 mM Tris-HCl, pH 7.4, 2 mM magnesium chloride, 140 mM sodium chloride, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 0.1% tritonX100), and stored at -70°C. The samples were thawed at 37°C in a water bath. The nuclear extract was prepared as described previously (Singh & Aggarwal, 1995). NFkB and AP-1 double standard oligonucleotides (Promega, Madison, WI) were end-labeled with [ $\gamma$ -<sup>32</sup>P]-ATP (Amersham, Arlington Heights, IL). One microliter (30,000 cpm) of labeled probe was incubated with 3  $\mu$ l of nuclear extract (protein concentration, 5  $\mu$ g) and binding buffer (15 mM Tris-HCl, pH 7.5, 7.5% glycerol, 75 mM sodium chloride, 1.5 mM EDTA, 1.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.3% NP-40, and 20  $\mu$ g bovine serum albumin), final volume 25  $\mu$ l for 30 min at 25°C and separated with a 4% nondenaturing polyacrylamide gel for 1.5 h with 0.5% TBE (Singh & Aggarwal, 1995). The gels were vacuum dried for 1 h and exposed to X-ray film for 12 h at -70°C. The developed autoradiograms were densitometrically quantified with an Alphaimager 2000 version 4.03 (Innotech Corp, San Leandro, CA) video image analyzer. The specificity of shifted bands was confirmed by incubating first with a 10-fold excess of cold oligonucleotides and then with radioactively labeled oligonucleotides. Results are expressed as percentage of positive control (100%, PMA and PHA stimulated nuclear extracts from normal, nonstressed individuals) for each gel.

Sixteen women,  $52.1 \pm 3.7$  years of age, were enrolled from the Loyola University Breast Care Center on the day that their physician recommended the need for a breast biopsy. After signing an informed consent, blood samples (16 ml) were obtained by venipuncture and the psychological assessments were administered in a private area adjacent to the clinic. At approximately 5-7 days after T1, the study women underwent breast biopsy. These same women were also sampled post biopsy (T2). T2 occurred 7-10 days after the women learned the results of their breast biopsy. At this time the women met with the study's nurse researcher at the Breast Care Center.

TABLE 1  
Evaluation of Psychological Stress in Breast Biopsy Patients

Psychological test	T1. Prebiopsy	T2. Postbiopsy	Normal controls
POMS	22.0 $\pm$ 7.1 <sup>a</sup>	0.2 $\pm$ 6.9	2.2 $\pm$ 9.3
STAI	42.7 $\pm$ 4.4 <sup>a</sup>	27.8 $\pm$ 2.2	27.2 $\pm$ 1.7

*Note.* Values represent mean  $\pm$  standard error of mean. POMS, Profile Of Mood State; STAI, State Trait Anxiety Inventory; T1, Time 1, prior to breast biopsy; T2, Time 2, 7–10 days after breast biopsy results were known.

<sup>a</sup> Statistically significant  $p < .05$  from T2 and controls.

their blood was collected, and the psychological instruments were administered. Age-matched, nonstressed control women were solicited from within the University community. At a convenient time these women were met in a University office setting. In a procedure similar to that for the biopsy group, their blood was obtained by venipuncture and the same psychological instruments were administered. Control women were sampled at one time point only.

For the purposes of this study, anxiety was assessed using Spielberger's State Anxiety Inventory (STAI) and mood was assessed using the Profile of Mood States (POMS). The STAI measures the state of anxiety and it is designed to assess "state of mind" or anxiety at the moment. The STAI has alpha reliability coefficients of 0.83–0.92 and convergent validity with other anxiety instruments (alpha 0.75–0.80). The POMS is a reliable and valid tool designed to identify and assess general distress and mood. It has internal consistency reliabilities of 0.87–0.95, and stability coefficients (test–retest) of 0.65–0.74 (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1970; McNair, Lorr, & Droppleman, 1992). These instruments were used to reflect the level of psychological distress experienced by these women pre and post breast biopsy. This study was approved by the Institutional Review Board for the study of Human Subjects of Loyola University Medical Center.

## RESULTS

Table 1 shows the psychological profile of women pre biopsy (T1) and post biopsy (T2). Mood disturbance and anxiety, as measured by the POMS and STAI, showed a significant ( $p < .05$ ) decrease from pre- (T1) to postbiopsy (T2). These results demonstrated that the women experience two forms of psychological distress, anxiety and mood disturbance, at T1. At T2, reduced anxiety and improved mood were demonstrated and were not different from nonstressed, age-matched, control women. Electrophoretic mobility shift assays were used to quantify the AP-1 and NFkB activity in the stimulated peripheral blood lymphocytes of the subjects. Lymphocytes obtained at T1 exhibited lower expression of nuclear NFkB and AP-1 compared to T2 (Table 2). No change in nuclear localization of another transcription factor, Oct-1, was noted either before or after biopsy. Examples of the electrophoretic mobility shift assays are presented in Figs. 1, 2, and 3. The reduction in expression of AP-1 and NFkB was greater for NFkB than AP-1. Follow-up of these patients at the post-biopsy period (T2) showed NFkB and AP-1 activity to be higher ( $p < .001$ ) than that observed at T1. From T1 to T2 the NFkB activity increased nearly threefold, while that of AP-1 nearly doubled. No phenotypic differences were observed in the

TABLE 2  
Evaluation of Peripheral Blood Lymphocytes of Breast Biopsy Patients

Transcription factor	Percentage of Positive Control		Normal controls
	T1, Prebiopsy	T2, Postbiopsy	
NFkB	32.8 $\pm$ 5.3 <sup>a</sup>	104.3 $\pm$ 3.2	105.9 $\pm$ 3.8
AP-1	57.4 $\pm$ 5.1 <sup>a</sup>	107.8 $\pm$ 2.7	94.7 $\pm$ 5.1
Oct-1	101.8 $\pm$ 4.2	102.6 $\pm$ 3.9	100.9 $\pm$ 5.0
Lymphocyte Population %			
T	71.8 $\pm$ 9.0	72.2 $\pm$ 8.1	70.7 $\pm$ 5.6
B	16.1 $\pm$ 5.1	14.7 $\pm$ 4.8	17.8 $\pm$ 5.4
NK	13.0 $\pm$ 11.7	12.6 $\pm$ 9.2	11.3 $\pm$ 6.6

*Note.* Percentage of AP-1 and NFkB activity in the nuclear extracts of peripheral blood lymphocytes stimulated with PMA/PHA for 48 hrs. Decreased NFkB and AP-1 activity were observed prebiopsy (T1). When the stress was relieved, postbiopsy (T2), AP-1, and NFkB nuclear activity increased. A positive normal (nonstressed) control (100% activity) was examined for each set of samples for calculating relative percentage activity. Values represent mean  $\pm$  standard error of mean. No statistically significant differences were observed in the percentage of Oct-1 nuclear activity. No statistically significant differences were observed between the percentages of the individual lymphocyte populations analyzed between groups.

<sup>a</sup> Statistically significant  $p < .001$  when compared to T2 and normal controls.

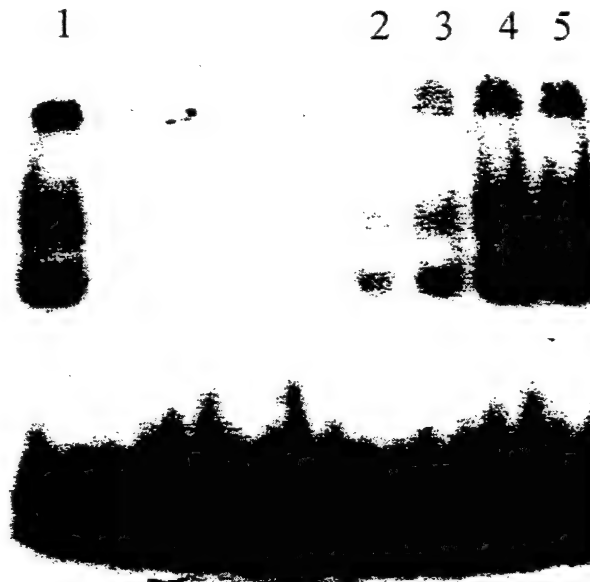
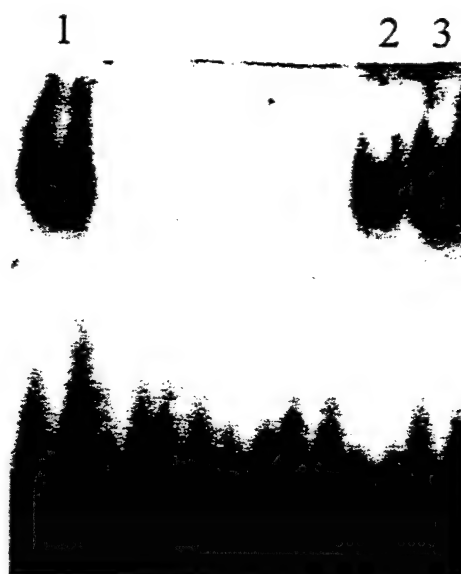
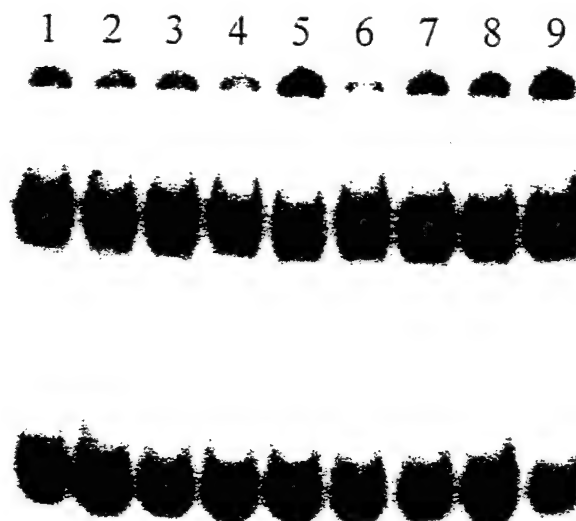


FIG. 1. Representative electrophoretic mobility shift assessment of NFkB nuclear localization in extracts from the lymphocytes of a woman pre- and postbiopsy. Lane 1 contains the PMA and PHA stimulated nuclear extract from the lymphocytes of a normal, nonstressed individual. Lanes 2 and 3 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of a woman prior to biopsy. Lanes 4 and 5 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of the same woman postbiopsy. Lanes 2-5 are extracts derived from the lymphocytes of one woman. Densitometric quantification of the autoradiographic results were calculated for both of the bands in each lane. The results for the higher molecular weight band are presented in Table 2.



**FIG. 2.** Representative electrophoretic mobility shift assessment of AP-1 nuclear localization in extracts from the lymphocytes of a woman pre- and postbiopsy. Lane 1 contains the PMA and PHA stimulated nuclear extract from the lymphocytes of a normal, nonstressed individual. Lane 2 contains the PMA and PHA stimulated nuclear extract from lymphocytes of a woman prior to biopsy. Lane 3 contains the PMA and PHA stimulated nuclear extract from lymphocytes of the same woman postbiopsy.



**FIG. 3.** Representative electrophoretic mobility shift assessment of Oct-1 nuclear localization in extracts from the lymphocytes of women pre- and postbiopsy. Lane 1 contains the PMA and PHA stimulated nuclear extract from the lymphocytes of a normal, nonstressed individual. Lanes 2, 3, 4, and 5 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of women prior to biopsy. Lanes 6, 7, 8, and 9 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of women postbiopsy. Lanes 2-9 are extracts derived from the lymphocytes of four women and are presented sequentially with the following exception: Lanes 2, 4, 6, and 8 are postbiopsy extracts from the same woman.

women's lymphocyte populations from T1 to T2. The activity of the transcription factors in women after the experience of breast biopsy was similar to that of age-matched and nonstressed women.

### DISCUSSION

We have examined nuclear localization of two transcription factors, NFkB and AP-1, in lymphocytes isolated from the peripheral blood of women scheduled to undergo biopsy for suspected breast cancer. The blood samples were collected at two different time intervals, before and after breast biopsy. The prebiopsy sampling occurred on the day the woman's physician informed her of the need for a breast biopsy. At this time the women exhibited increased anxiety and mood disturbance, which was most likely related to the uncertainty associated with an impending biopsy of the breast. From pre to postbiopsy there was a clear decrease in the anxiety and mood disturbance expressed by these women. These changes in anxiety and mood disturbance were reflected by changes in NFkB and AP-1. The nuclear levels of these transcription factors were decreased prebiopsy or during the time of heightened psychological stress; while at postbiopsy both the psychological stress and the transcription factors returned to that of nonbiopsied control women.

Experimental studies in animals and clinical studies in humans suggest that psychological stress decreases immune function and increases the risk for infectious disease and the reactivation of latent viruses. NFkB regulates the expression of gene products such as cytokines, acute phase proteins, and adhesion molecules. These are necessary components of immunity which ensure effective protection from infectious disease (Dobbin, Harth, McCain, Martin, & Cousin, 1991; Bonneau, Zimmerman, Ikeda, & Jones, 1998; Mae, Song, Lina, Jongh, Van Gastel, Kenis, Bosmans, De Meester, Benoy, Neels, Demedts, Janca, Scharpe, & Smith, 1998; Kopp & Ghosh, 1995). It is possible that a stress-induced decrease in NFkB and AP-1 activity may down regulate the secretion of important cytokines that promote an effective immune response and are required for optimal protection from infection. This is especially important for cancer patients undergoing immunosuppressive chemotherapy. Decreased immunity in cancer patients may not only increase susceptibility to infection but may also accelerate the growth and progression of cancer (Cohen & Rabin, 1998). In a recent study, reduced translocation of lymphocyte transcription factors was observed in women with breast cancer. Those authors speculated that a defect in transcription factor translocation may be related to breast cancer (Kurt, Urba, Smith, & Schoof, 1998). Although the design of this study does not permit a causal interpretation, the data does suggest that the emotional distress associated with impending breast biopsy may influence the capacity of lymphocytes to translocate nuclear transcription factors. Therefore, psychological stress may be an important modulator of transcription factors which, in turn, may result in reduced immune function at the level of transcription.

NFkB, AP-1, and other transcription factors act together to regulate and activate components of the immune system. To our knowledge, this is the first report suggesting a possible molecular mechanism whereby psychological stress may modulate immune function. Because NFkB, AP-1, and related transcription factors play a central role in the immune system (Kopp & Ghosh, 1995; Thomas, Tymms, McKinlay, Shannon, Seth, & Kola, 1997), they may be a marker or means by which to measure the overall effect of stress upon immune function.

## ACKNOWLEDGMENTS

Supported in part by USPHS NR-00085 and AI-31127, the Department of the Army DAMD 98-8120, and by the Cancer Federation. The content of this manuscript does not reflect the position or the policy of the Department of the Army or the government.

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Received September 10, 1999.

## CHAPTER 3

# Stress, Immunity, and Health Outcomes

*Linda Witek-Janusek and Herbert L. Mathews*

**T**he assumption that psychological stress, physical stress, mood, and behavior modulate the immune system, and predispose an individual to illness, is centuries old. In the sixteenth century, the Greek physician Galen observed that melancholy women were more predisposed to the development of tumors. Today, the assumption is widely held that stress, emotions, and behavior affect health, well-being, and predisposition to disease. For example, a character proclaims in Woody Allen's film *Manhattan*, "I can't express anger, I grow a tumor instead." Only recently, however, has this mind-immune relationship been subjected to rigorous scientific inquiry.

The organized establishment of the science of psychoneuroimmunology is often

credited to Robert Ader, who first introduced this term in his presidential address to the American Psychosomatic Society (Ader, 1980). Ader defined *psychoneuroimmunology* as the study of the interactions among behavioral, neural, endocrine (neuroendocrine), and immunological processes of adaptation. The central premise is that an individual's response and adaptation to the environment is an integrated process involving interactions among the nervous, endocrine, and immune systems. This is in contrast to the traditional view of the immune system in which it is autonomous and functions independently of the other organ systems of the body. Today, psychoneuroimmunology is a multidisciplinary science that includes nurses, psychologists, immunologists, microbiologists, neuro-

**AUTHORS' NOTE:** This chapter is dedicated to the memory of my (LWJ) mentor and my friend, Dr. Sabath F. Marotta (1929-1996), who introduced me and numerous other nurses to scientific inquiry and stress physiology. May his memory live on in our collective contributions to the field of stress. This work was supported in part by the Department of the Army (DAMD-98-8120), the National Cancer Institute (CA-77120-01), the National Institute of Nursing Research (NR-00085), the National Institute of Allergy and Infectious Disease (AI-31127), Catholic Health Partners, and the Cancer Federation. The expertise of Josh Takagishi and Maribel Barrigan is gratefully acknowledged. The content of this chapter does not reflect the position or the policy of the Department of the Army or the U.S. government.

scientists, endocrinologists, and others. The collective aims of these scientists are to explore and explain the common belief that one's behavior and emotions can influence stress, immunity, and health outcome.

Despite the recent development of psychoneuroimmunology as a discipline, initial evidence that linked stress to the immune system was reported by Hans Selye in the 1930s. In his general adaptation syndrome, Selye described a triad of responses to acute physical stress that consisted of adrenal gland enlargement, gastric erosion, and thymic involution (Selye, 1936, 1976). Since then, scientific evidence confirming biological links among the nervous, endocrine, and immune systems has accumulated. These links include direct innervation of lymphatic tissue by the central nervous system and a shared communication network in which cells of the nervous, endocrine, and immune systems use common molecules and receptors to reciprocally modulate biologic activity. Thoughts, emotions, and behavior are known to activate anatomical and biochemical pathways, and these pathways in turn modulate immune function (La Via & Workman, 1998). Such observations and demonstrations have permitted advocates of psychoneuroimmunology to suggest that biobehavioral interventions aimed at strengthening immunocompetence may be an important component of holistic health care (Kiecolt-Glaser & Glaser, 1992).

#### ► NEURAL-IMMUNE INTERACTIONS

The connection between the brain and the immune system is through direct innervation of lymphoid tissue and through the release of products from the brain that bind to membrane receptors on immunologically competent cells. It is clear that primary and secondary lymphoid tissues are innervated with noradrenergic and peptidergic nerve fibers (Felten et al., 1987). The Felten's immunohistochemical studies provide direct evidence of the close association between presynaptic

sympathetic nerve endings and lymphocytes and macrophages (Felten, Felten, Carlson, Olschowka, & Livnat, 1985). Experimentally produced brain lesions of the hypothalamus, hippocampus, and cerebral cortex alter immune function, suggesting a neural-immune interactive network of connections. Those areas of the brain that exert immunomodulatory effects are areas concerned with emotions and with visceral, autonomic, and neuroendocrine regulation, thus establishing the "hardwiring" between neural centers that process emotions and immune cells. Further verification of a neural-immune network or axis was provided when lymphocytes and macrophages were shown to bear receptors for adrenergic substances (both  $\alpha$ - and  $\beta$ -adrenergic receptors) and various neuropeptide hormones, including vasoactive intestinal polypeptide, somatostatin, calcitonin gene-related peptide, substance P, and opioids (Stevens-Felten & Bellinger, 1997). The presence of such receptors on immune cells provides a mechanism whereby the immune system can respond to biochemical signals from the brain. Activation of these receptors leads to functional changes in immune response (i.e., lymphocyte proliferation, cytotoxicity, antibody production, and cytokine secretion).

A pivotal step in firmly establishing that the brain and immune system interact was accomplished by psychologists who, using animal models, demonstrated that classical psychological (Pavlovian) conditioning could produce immunologic changes (Ader & Cohen, 1993). Such conditioning and its effect on the immune system have been demonstrated clinically. For example, research has documented the occurrence of anticipatory immunosuppression prior to the administration of chemotherapy (Bovbjerg et al., 1990; Fredrikson, Furst, Lekander, Rothstein, & Blomgren, 1993).

Investigators continue to unravel the intricate interplay among the nervous, endocrine, and immune systems. The associated immunologic changes that occur in response to neuroendocrine mediators, however, are

highly complex, and often the characterization of putative interactions has been measured only *in vitro*, in which one variable is manipulated. *In vivo*, however, immunologically competent cells respond to multiple stimuli, including numerous so-called molecules of emotion, within a microenvironment. Ultimately, the net immune response is an integration of these stimuli. The multiple levels and complexity of such immune modulation are remarkable, considering that numerous peptide and hormonal mediators can augment and diminish immune function (Wang, Fiscus, Yang, & Mathews, 1995; Witek-Janusek & Mathews, 1999a). It remains to be determined how these peptides and mediators fit within a homeostatic framework or are altered by environmental perturbation.

Adding additional complexity, it is well established that not only do nerves and secretory products from the brain influence immune function but also the converse is true. Immune activation can modulate central nervous system activity. Hugo Besedovsky and collaborators conducted seminal studies, which demonstrated that antigenic challenge of the immune system can produce an increase in neural firing within the medial hypothalamus (Besedovsky, Felix, & Haas, 1977). A peak immune response was associated with a decrease in norepinephrine turnover. Cytokines produced by antigen-activated lymphoid cells altered the turnover of norepinephrine (Besedovsky, del Rey, Prada, Burri, & Honegger, 1983).

It is now understood that alterations in cytokine secretion subsequent to immune activation mediate behavioral effects often associated with illness. For example, interleukin-1 (IL-1), IL-6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) mediate sickness behavior (the fatigue, lethargy, and decreased appetite associated with infectious illness) (Dantzer et al., 1998). Because they are large protein molecules, cytokines do not readily cross the blood-brain barrier. They are believed to signal the brain by entering neural structures that do not possess tight capillary endothelial barriers, such as the organum vasculosum

laminae terminalis and area postrema. In addition, recent evidence indicates that cytokines released in the periphery can activate sensory afferents, such as vagal afferents, and signal central nervous system (CNS) areas involved in immune-related behavioral responses (Watkins, Meier, & Goehler, 1995).

Collectively, this evidence supports the concept of a dynamic neuroendocrine-immune network whereby soluble products of immunologically competent cells affect the CNS following antigenic challenge. It is this conceptualization that led Blalock to liken the immune system to a sensory organ capable of informing the CNS of an antigenic challenge (Blalock & Smith, 1985).

Both psychological and physical stressors are known to activate neuroendocrine pathways that interact with the immune system (Chrousos, 1998). Stressor activation leads to increased secretion of neurosecretory hormones from the hypothalamus, such as corticotropin-releasing hormone (CRH). In turn, these hypothalamic hormones regulate secretion of pituitary hormones, such as adrenocorticotropin hormone (ACTH) and endorphins. Because there are shared hormonal receptors on cells of the immune and neuroendocrine systems, reciprocal interactions between these systems are possible (Reichlin, 1993; Weigent & Blalock, 1999). Neuroendocrine secretory products have immunomodulatory effects and alter leukocyte function (e.g., the immunologic effects of glucocorticoids, endorphins, ACTH, growth hormone, and prolactin). These effects include the regulation of cytokine secretion, antibody synthesis, natural killer cell (NK) activity, and lymphocyte proliferation (Weigent & Blalock, 1999). The complex interactions between the neuroendocrine and immune systems are believed to, in part, downregulate inflammatory responses and limit continuous proliferation of lymphoid cells or excessive production of immune cell products or both (Munck & Guyre, 1986).

Interestingly, neuroendocrine hormones can be produced by leukocytes, the most well studied of which is proopiomelanocortico-

trocin (POMC). POMC is a precursor molecule for the hormones ACTH and endorphin. Although the role of hormones produced by immune cells is under investigation, it is likely that they function by autocrine/paracrine mechanisms within the local lymphoid microenvironment (Weigent & Blalock, 1999). The finding that immune cells can produce hormones normally secreted from the anterior pituitary emphasizes the close relationship of the immune and endocrine systems. Finally, immune cell secretory products (e.g., cytokines) alter neuroendocrine cell secretion. For instance, cytokines have actions at both the hypothalamic and pituitary levels. Cytokines, such as IL-1, IL-2, IL-6, and TNF, activate the adrenal axis, whereas IL-1 and TNF inhibit the gonadal axis and TNF and interferon-gamma (IFN- $\gamma$ ) suppress the thyroid axis (Weigent & Blalock, 1999).

The bidirectional nature of the neuroendocrine and immune systems likely accounts for the effect of stress on the immune system (Figure 3.1). Regulatory hormones and neuropeptides once believed to be confined to the brain or endocrine system or both are now known to be mutually expressed by all three systems (nervous, endocrine, and immune), and as a result each system may be capable of modulating the function of the other.

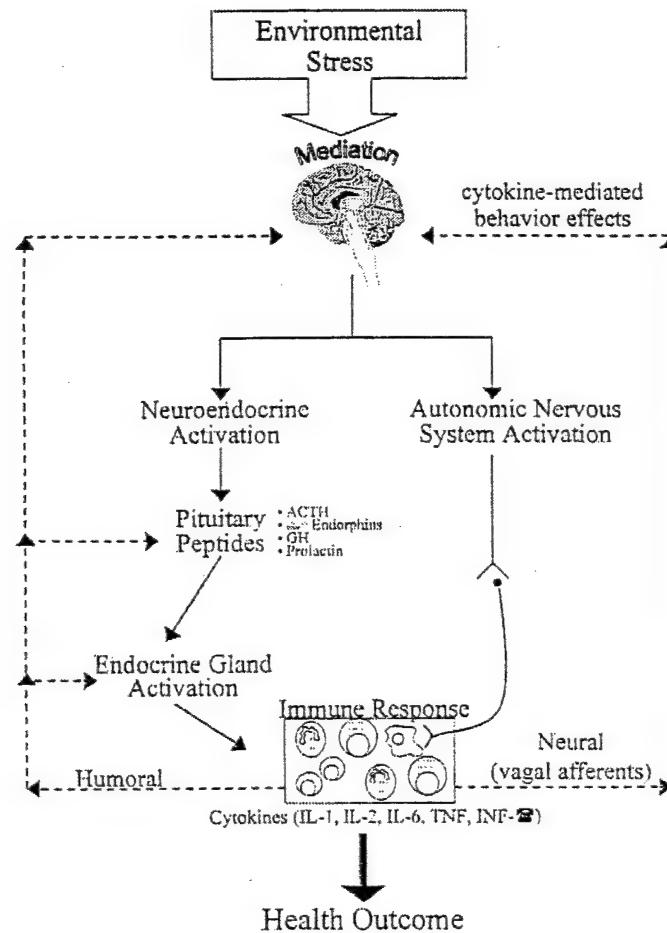
In summary, during the past 15 years empirical evidence has emerged that supports the existence of a communication network linking the nervous, endocrine, and immune systems. Psychological stimuli modulate the immune response either through direct activation of neural pathways that terminate in lymphoid tissue or by activation of neuroendocrine circuits leading to the release of molecules that bind to immunologically competent cells. Conversely, the immune system recognizes noncognitive stimuli, such as bacteria, fungi, and viruses, resulting in the secretion of an array of cytokines that act on receptors of the neuroendocrine system. Collectively, cognitive and noncognitive stimuli form a network, which is the basis for behaviorally induced al-

terations in immune function (Weigent & Blalock, 1999). It is likely that this neuroendocrine-immune network mediates the effect of stress on the development or progression or both of immune-based disease.

## ► STRESS AND IMMUNITY

Stressful life events, and the subsequent emotional and behavioral responses to these events, are commonly believed to alter immunity. When external demands (i.e., stressors) exceed an individual's adaptive capabilities, a stress response ensues (Lazarus & Folkman, 1984). It is the subsequent neurological and endocrinological changes that are believed to produce stress-elicited immune alterations. Studies during the past decade provide convincing evidence that psychological stress can affect the immune system (e.g., lymphocyte proliferation, NK activity, antibody synthesis, and cytokine production). These studies have been accomplished with animal models and in human stress situations, including both experimentally produced stress and naturalistic paradigms for stress evaluation. This chapter focuses on the major human stress paradigms.

Early studies supporting the effects of stress on immunity were conducted by the research team of Glaser and Kiecolt-Glaser. These investigators conducted a series of stress studies in medical students that demonstrated the immunosuppressive effects of in-class examinations (Kiecolt-Glaser, Garner, Speicher, Penn, & Glaser, 1984). The results of these studies indicate that the stress that accompanies examinations leads to a wide range of immunosuppressive effects, including decreased NK cell activity (Glaser, Rice, & Speicher, 1986), lymphocyte proliferation (Glaser et al., 1987), IFN- $\gamma$  production (Glaser et al., 1986), IL-2 production (Glaser et al., 1990), and latent viral activation as evidenced by increased antibody titer to the virus (Glaser et al., 1992).



**Figure 3.1.** Summary and possible interconnections by which environmental stimuli, or stress, can affect the immune response and health outcomes. Perceived environmental stress is mediated by the central nervous system and can lead to neuroendocrine and autonomic nervous system activation. As a result, the immune response can be altered by autonomic nerve fibers that directly synapse with immune cells and by circulating catecholamines released from the adrenal medulla. In addition, further alteration can be produced by secretory products (hormones and neuropeptides) released from the pituitary and endocrine target glands (adrenal cortex, thyroid, ovaries, and testes). In turn, feedback (dashed lines) from immune cell products (cytokines) can modulate endocrine and central nervous system activity by either humoral or neural communication networks.

Furthermore, medical students with lower anxiety levels had faster and stronger immune responses to hepatitis B vaccination than did students with higher levels of anxiety (Glaser et al., 1992; Glaser, Kiecolt-Glaser, Malarkey, & Sheridan, 1998). Recently, examination stress was shown to alter cytokine

production that shifts the cytokine pattern away from a Th1 to a Th2 type of response (Maes et al., 1998). This shift is characterized by a decrease in secretion of IFN and an increased secretion of IL-10. The authors suggest that the shift in cytokine production may partially explain the increased incidence of

viral infection, latent viral expression, allergic and asthmatic reactions, and autoimmunity reported during times of high stress (Marshall et al., 1998).

The type of immune response seen as a result of stress is dependent on the acute versus chronic or repeated nature of the stressful event (McEwen, 1998). For example, acute stressors, such as parachute jumping, are correlated with a mobilization in the numbers of NK cells; this is likely attributable to a change in cell trafficking related to adrenergic arousal (Schedlowski et al., 1993) or glucocorticoid secretion (McEwen, 1998) or both. Studies such as these suggest that acute stress produces a redistribution of lymphocytes and macrophages in the body. These cells marginate on blood vessel walls and compartmentalize in the skin, lymph nodes, and bone marrow. It is theorized that acute stress activates the immune response and prepares the organism for potential encounters with an immunologic challenge. This activation may exacerbate autoimmune or allergic responses (Dhabhar, Miller, McEwen, & Spencer, 1996). Repeated or chronic stress, however, suppresses immune responsiveness, particularly cell-mediated immunity, and increases susceptibility to infectious challenge and tumor cells (McEwen, 1998). Chronic stressors, such as bereavement, caregiving, marital conflict, and divorce, impair the ability of NK cells to be lytic and to respond to cytokines (IFN- $\gamma$  or IL-2) *in vitro* (Esterling, Kiecolt-Glaser, & Glaser, 1996; Herbert & Cohen, 1993; Kiecolt-Glaser, Dura, Speicher, Trask, & Glaser, 1991; Kiecolt-Glaser, Glaser, Cacioppo, & Malarkey, 1998). Other aspects of cellular immunity are also affected by chronic stress (Herbert & Cohen, 1993), including decreased lymphoproliferation, NK cell activity, numbers of circulating lymphocytes, as well as salivary and serum immunoglobulin levels.

The impact of chronic stress has been poignantly illustrated by assessing the immune response of individuals caring for relatives with Alzheimer's disease. Kiecolt-Glaser et al. (1987) found that such caregiving was accompanied by greater distress and

heightened levels of herpes virus-specific antibody (suggesting viral reactivation). Furthermore, elderly individuals experiencing the chronic stress of caring for a spouse with Alzheimer's disease had attenuated responses to the influenza vaccine and more physician-confirmed respiratory infections than control subjects. Health behaviors did not differ between the two groups.

Conversely, Irwin et al. (1991) reported no differences in NK cell activity between caregivers and control subjects. Esterling et al. (1996), however, found that both caregivers and former caregivers (those whose relative had died at least 2 years previously) had blunted NK cell activity compared to nonstressed control subjects. Interestingly, the results of this study suggested that the psychological and immunological aftermath of caregiving persists beyond the actual stressful experience. In an attempt to reverse the immunosuppressive effects of stress in the elderly, these investigators enrolled subjects in a 1-month stress-reduction program that used progressive muscle relaxation. This form of stress reduction produced a 30% increase in NK cell activity (Kiecolt-Glaser et al., 1985).

The type and magnitude of stress-elicited effects on the immune system are influenced by many factors. Such factors may relate to the stressor, such as the type, intensity, and duration of the stressful stimulus. The sampling time frame between the stressor and the immune response can also influence whether an effect can be measured. Furthermore, not all components of the immune system may respond to a stressor. Therefore, it is important that the immune parameter to be measured be carefully chosen within the context of the population or illness studied or both. A variety of host or subject factors will also influence the immune response to stress, such as age, preexisting illness, nutritional status, substance abuse, exercise habits, adequacy of sleep, coping, and social support (Kiecolt-Glaser & Glaser, 1988; Zeller, McCain, McCann, Swanson, & Colletti, 1996).

The primary criticism of many stress-immune studies is that although the immune change observed is often statistically signifi-

cant, the magnitude of the change is small and often within normal limits. Whether or not such a change in immune function is significant to health outcomes remains to be determined. There are studies, however, that have found that stress-induced immune changes can increase susceptibility to infectious disease and may also influence the course of disease (Cohen, Tyrel, & Smith, 1991; Spiegel, Bloom, Kraemer, & Gottheil, 1989). Such studies support the contention that even small changes in immune function may have health-related significance.

### ➤ STRESS-IMMUNITY AND HEALTH OUTCOMES

One fundamental question that remains unanswered in the field of psychoneuroimmunology is whether or not stress-induced alteration in immune function plays a role in disease development or disease progression or both. Numerous studies, although inconclusive, have shown stress to influence the course or progression of illness or disease (e.g., cancer, infectious disease, and HIV). Few studies, however, provide definitive evidence that links stress, immunity, and health outcomes. This area remains a challenge for researchers in psychoneuroimmunology.

One of a handful of well-controlled studies that examined the effect of psychological stress on susceptibility to illness was conducted by Cohen et al. (1991). They investigated the relationship between stress and the common cold using a viral challenge paradigm. Following extensive health and psychological assessment (for the previous 12 months), 394 volunteers were randomized to receive either a low infectious dose of a respiratory virus or saline. For 2 days prior to viral challenge and 7 days postchallenge, volunteers were quarantined. Rhinovirus infection was based on the development of clinical symptoms of a cold, the development of virus-specific antibodies, and the culture and isolation of the inoculated virus. The results revealed that psychological stress predicted susceptibility to colds among the initially healthy

people exposed to the respiratory virus. Psychological stress was operationalized as an index of the number of negative life events, the perceived impact of these negative life events, perceived stress, and negative affect. In a related study, Cohen et al. analyzed the relationship of an individual's social contacts to the development of the common cold. In 276 volunteers exposed to rhinovirus, a greater resistance to upper respiratory infection was exhibited in subjects who had the greatest diversity of social contacts (friends, family, and community). Interestingly, greater resistance to infection was related to increased numbers of social contacts and not to the absolute number of individuals involved in the social contacts (Cohen, Doyle, Skoner, Rabin, & Gwaltney, 1997). Recently, these investigators reported that acute stressful life events (less than 1 month in duration) were not associated with the onset of colds. Severe chronic stressors (1 month or longer in duration), however, were associated with the risk of cold development. The most prevalent chronic stressors for this study group were under- or unemployment or enduring interpersonal difficulties with family or friends (Cohen et al., 1998).

### ➤ STRESS AND WOUND HEALING

Studies of the effects of stress on wound healing and tissue repair have suggested that stress-induced neuroendocrine activation impairs healing and delays recovery. Both animal and human models of wound healing have been used to examine the effects of stress. In one study (Padgett, Marucha, & Sheridan, 1998), the effects of restraint stress on the healing of a sterile punch wound in rats were studied. Rats were subjected to restraint stress for 3 days prior to and for 5 days after wounding. Wound healing was measured using photography and image analysis. Compared to control rats, which were wounded but not restrained, healing was delayed an average of 3 days in the restraint stressed group. Treatment of the restraint stressed group with a glucocorticoid receptor

antagonist produced healing rates that were similar to those of control animals. These results demonstrate that restraint stress delayed wound healing. Because the glucocorticoid antagonist reversed this effect, the delay was likely due to a stress-induced increase in glucocorticoids. Padgett et al. (1998) hypothesized that the stress-induced elevation in glucocorticoids prevented the early part of wound healing in which macrophages move into the area to remove cellular debris and secrete growth factors, cytokines, and chemotactic factors needed for tissue repair. Glucocorticoids are well-known to suppress the inflammatory response, including the production of IL-1, IL-6, and TNF- $\alpha$  (Bendrup, Hilton, Meager, & Hamilton, 1993). The results of this study provide compelling evidence, albeit in an animal model, that disruption of neuroendocrine homeostasis by a stressor modulates wound healing.

The ability of stress to delay wound healing has also been shown in human stress paradigms. Kiecolt-Glaser, Marucha, Malarkey, Mercado, and Glaser (1995) studied the effects of chronic stress on caregivers (spouses) for patients suffering from Alzheimer's disease. Punch biopsy wounds were applied to caregivers and age-matched control subjects. The results indicated that wound healing was markedly delayed in the caregivers compared to control subjects. These differences were not related to other covariates, such as nutrition, sleep, or the presence of other illnesses. In another study, wound healing was delayed by the more acute and benign stress of academic examinations. Two punch biopsy wounds were placed on the hard palate of dental students during summer vacation and then on the contralateral side 3 days prior to the first major exam of the term. Mucosal wound healing took 3 days longer to complete during the exam period. The production of mRNA for IL-1 $\beta$  was also reduced during the stress of examination (Marucha, Kiecolt-Glaser, & Favagehi, 1998).

In summary, the previously discussed studies provide compelling evidence suggesting that stress can impair tissue repair and

wound healing. This can have significant implications for recovery from injury and surgery, especially in vulnerable populations, such as individuals with diabetes, impaired tissue perfusion, and advanced age. Delayed healing of wounds increases the risk for wound complication by infectious pathogens, which can further prolong recovery and length of hospital stay. Nurses are in a pivotal position to recognize and reduce stress and to teach stress management skills. This has the potential to promote both healing and recovery and enhance health outcomes.

### ► STRESS-IMMUNITY AND CANCER

The immune system is believed to play a role in surveillance against malignantly transformed cells. It has been hypothesized that stress-induced suppression of immune cell activity (e.g., NK cell activity) may alter the clinical course of cancer. The relationship between NK cell activity and cancer is complex (Rosenberg & Lotze, 1986). NK cells, however, are also important in the control of viral infections (Trinchieri, 1989; Whiteside & Herberman, 1989). As such, NK cells may prevent the development of infectious complications in cancer patients who are often immunosuppressed.

There are a few highly intriguing studies that have examined the relationship between stress and immunity in cancer patients. Levy et al. (1990) found that estrogen receptor status predicted NK cell activity in 66 women with Stage I or II breast cancer 3 months after surgery with or without adjuvant therapy. These researchers also showed that social support contributed significantly to a regression model predicting higher NK cell activity. That is, the greater an individual's social support, the higher the individual's NK cell activity.

Andersen et al. (1998) studied stress-immune parameters in 116 women who were diagnosed with invasive breast cancer (Stages II and III). Women were enrolled within 4 months of their breast surgery but prior to

adjuvant therapy initiation. Stress was measured using the Impact of Event Scale, which is a self-report measure of intrusive and avoidance thoughts and behaviors (Horowitz, Wilner, & William, 1979). Using hierarchical multiple regression analysis, their results revealed that higher stress levels significantly predicted lower NK cell activity, diminished NK cell response to IFN- $\gamma$ , and decreased lymphocyte proliferation (Andersen et al., 1998). It is noteworthy that this study controlled for extraneous variables that might also affect immunity, including age, stage of disease, nutritional status, and days since surgery. The results are intriguing and suggest that stress may play a pivotal role in women with cancer, possibly resulting in more susceptibility to cancer progression or infectious complications or both.

Researchers have begun to address the definitive question as to whether psychosocial interventions can produce health effects that slow cancer progression and promote survival (Fawzy, Fawzy, Arndt, & Pasnau, 1995; Greer & Brady, 1988; Spiegel, 1996). Randomized prospective trials have shown protective effects of psychosocial interventions on cancer progression (Spiegel, Sephton, Terr, & Stites, 1998). Fawzy et al. (1993) studied the effects of a behavioral intervention in patients with malignant melanoma. Subjects were randomized to an intervention consisting of six 90-minute sessions including health education, stress management, coping skills, and group discussion. Six months later, the intervention group showed reduced psychological distress and enhanced immune function (increased IFN- $\alpha$  and augmented NK cell activity) compared to the nonintervention group. Although no association between survival and NK cell activity was found, individuals with higher baseline NK cell activity had a decreased incidence of disease recurrence.

In another study, the effects of a home visit and educational intervention program for lymphoma and leukemia patients were investigated (Richardson, Shelton, Krailo, & Levine, 1990). The results showed that patients in the intervention group were more compli-

ant with their medical treatment. More important, when controlling for this difference, members of the intervention group lived significantly longer than members of the control group.

A landmark study that assessed the effect of behavioral intervention on cancer survival was conducted by David Spiegel and colleagues (1989). They reported compelling results suggesting that an intervention, characterized as supportive-expressive group therapy, increased the survival of women with advanced breast cancer. Fifty of 86 women with advanced breast cancer were randomly assigned to support groups. The groups were designed to build strong supportive bonds, encourage "emotional expressiveness" about cancer, confront fears of dying and death, reorder life's priorities, improve relationships with family and friends, enhance communication with and development of shared problem solving with physicians, and teach self-hypnosis for pain control (Spiegel et al., 1998). The women were followed for 10 years, and a significant 18-month increase in survival for women in the intervention group was observed. Further analysis of the results of this study, in which medical records were reviewed, showed no difference in therapeutic treatment that could account for the differences in survival. Rather, a correlation was found between group support and survival. Spiegel's research team is currently replicating this study with a larger group in which endocrine markers of stress and cellular immune response, including NK cell activity, are being measured in addition to survival. It is hypothesized that psychosocial support will buffer the immunological consequences of cancer-associated stress and thereby improve disease outcomes (Spiegel et al., 1998).

In addition to the ongoing study of Spiegel, Andersen and colleagues (1998) are conducting a prospective, randomized study evaluating the effectiveness of stress-reduction interventions on psychological, immunological, and survival outcomes in women with advanced breast cancer. The structured intervention includes several stress-reduction strat-

egies, such as progressive muscle relaxation, social and emotional interventions designed to increase the quality of life, and healthy living habits. The intervention is provided weekly for the first 4 months and monthly for an additional 8 months. Psychological and immunological variables are being measured, with survival being the ultimate end point of this ongoing longitudinal study (McNeil, 1998; Voelker, 1997).

The role of psychological stress in cancer progression or response to treatment or both remains controversial, as was expressed in an editorial by Cohen and Rabin (1998). They contend that it is not clear if the effects of behavioral interventions are due to an individual's greater adherence to a healthy lifestyle or to the behavioral intervention therapy or both. The results of behavioral-based intervention studies are highly provocative and difficult to ignore, however. Indeed, the results of the ongoing clinical trials will provide further data that will aid in the understanding of the importance of stress, its impact on the immune system, and cancer control.

#### ► STRESS-IMMUNITY AND HIV

Individuals living with HIV face numerous stressors, such as family discord, change in occupation, economic hardship, social isolation, and bereavement (McCain & Zeller, 1996; Robinson, Mathews, & Witek-Janusek, 2000). Because the immune system plays a dominant role in the prevention of viral infections and in the suppression of latent viral infections, stress-induced changes in immune function may alter disease progression. Evidence suggests that stress-induced modulation of immunity may alter the course of HIV infection (McCain & Zeller, 1996; Robinson et al., 2000). Psychological variables are hypothesized to mediate host resistance to the HIV virus by modifying behavioral practices and by promoting an optimal neuroendocrine and immune milieu. Overall, most of these

studies are fraught with methodological difficulties, such as small and nonhomogeneous samples, lack of control for treatment and disease stage variables, inability to document or measure the presence of psychosocial stress in the sample, and lack of sensitive and relevant indices of immune measures. Nevertheless, the results are intriguing.

Goodkin, Fuchs, Feaster, Leeka, and Rishel (1992) studied stress-immune correlates in asymptomatic HIV-positive males. Although the sample size was small, the results suggested that men with a lower ability to cope with stress had lower total lymphocyte counts, whereas men with higher coping abilities had greater numbers of CD4+ T lymphocytes. A series of stress-immune studies have originated from the University of Miami's Center for the Biopsychosocial Study of AIDS; some of these studies have evaluated the psychoimmune effects of the stress of HIV antibody testing (i.e., test notification stress). This research team reported a significant relationship between increased anxiety (State Trait Anxiety Index [STAI]) at the time of notification of test results and decreased NK cell activity. No association with lymphocyte proliferation was found (Ironson et al., 1990).

In a similar study, during a 5-week period before and after HIV testing, seropositive subjects reported higher anxiety (STAI), higher depression, increased intrusive thoughts, and lower lymphocyte proliferation rates than seronegative subjects. Although plasma cortisol levels declined significantly in the seropositive group during the study period, they were within normal limits (Antoni et al., 1991). McCain and Cella (1995) found a significant relationship between high stress and lower CD4+ cell numbers in their study of a heterogeneous group (heterosexuals, minorities, injecting drug users, and those with various stages of disease progression) of 53 men with HIV disease. These same investigators examined the effect of a stress management intervention in HIV-positive individuals. Although a reduction in stress was demonstrated, they failed to show any significant ac-

companying change in immune function (McCain, Zeller, Cella, Urbanski, & Novak, 1996). In another intervention study, however, Esterling and colleagues (1992) measured antibody titers to Epstein-Barr virus (EBV) as the immune end point. Both HIV-positive and HIV-negative men in the 10-week program had significant decreases in anti-EBV viral encapsulated antigen when compared to their matched controls (Esterling et al., 1992). Because of the intriguing nature of these intervention studies, similar lines of research will likely be pursued in the future.

Although there is no clear mechanism for how stress influences HIV disease progression, Clerici and colleagues (1994) proposed an "immunoendocrinological" hypothesis implicating the potential role of elevated cortisol in the progression of HIV disease through modulatory effects on viral replication, cytokine modulation, and increased induction of apoptosis. Supportive evidence for this theory has been provided by reports that cortisol enhances HIV viral infections when added to cell culture medium containing human lymphocytes (Markham, Salahuddin, Veren, Orndorff, & Gallo, 1986) and HIV viral replication when added to monocyte cultures (Swanson, Zeller, & Spear, 1998). Norepinephrine, a major catecholamine released during stress, also accelerates HIV replication (Cole, Korin, Fahe, & Zack, 1998).

It is likely that studies examining psycho-neuroimmune parameters in HIV disease are limited by the immune outcome variables measured. It is possible that psychological effects may not have a measurable impact on indices of HIV disease development or progression or both. More important, stress may play an important role in the HIV-infected person's susceptibility to opportunistic infection. Consequently, there is a need to design and implement studies aimed at determining the role of psychological stress on immune system indices designed to measure defense mechanisms important in host defense against opportunistic infection (Robinson et al., 2000). The nature of the stress-immune relationship in HIV

disease needs to be carefully evaluated within the context of currently used antiretroviral therapy and within the context of future therapeutic approaches. Such therapies may not only alter immune responsiveness in those with HIV but also influence the type of stress they encounter as they live with HIV.

## ➤ STRESS-IMMUNITY AND INFECTION

Vulnerable populations, such as cancer patients and persons with HIV, face a multitude of stressors. These stressors can influence the immune system and increase susceptibility to infectious diseases. Psychological stress seems to alter the susceptibility of individuals to infectious agents and influences the onset, course, and outcome of the pathology associated with infection (Biondi & Zannino, 1997). Moreover, infectious disease can be a stressor. The human body's response to infection and to immunological challenge resembles both physical and psychological stress (Dunn, Powell, Meitin, & Small, 1989).

Infection can activate the hypothalamic-pituitary-adrenal axis (HPA) axis and increase the synaptic release of norepinephrine and serotonin in the brain (Dunn, 1993). Thus, by physiological criteria, infection can be regarded as stressful. The activation of the HPA axis associated with immune responses has been interpreted as a signal to the brain indicating the presence of an infectious threat from the external environment, triggering a stress response (Blalock & Smith, 1985). Once an effective immune response has been initiated, the HPA axis is thought to negatively regulate the immune system by the release of glucocorticoids that limit the inflammatory response and prevent overreactivity and auto-immune phenomena (Besedovsky, del Rey, Sorkin, & Dinarello, 1986; Munck & Guyre, 1986). Thus, the effects of stress on the immune system and the effects of the immune system on the neurologic response to infection are a complex and interrelated series of

physiologic events with many reciprocal interactions. Many specific infectious states appear to have a clear association with stress.

### *Tuberculosis*

Stress has long been associated with the pathogenesis of tuberculosis. With the recent resurgence of tuberculosis, understanding the potential role of stress in susceptibility to and progression of this infectious disease has become even more important. In previous studies, high rates of tuberculosis have been reported among socially isolated individuals and in schoolchildren and their teachers during periods of emotional stress, such as during war (Guyre, Girard, Morganelli, & Manganiello, 1988; Ishigami, 1919). These studies showed a reduced capacity of the infected individuals to phagocytize the infectious agent and suggested that stressful situations might serve as cofactors in the development of tuberculosis. Until recently, very little evidence existed to support this suggestion. Work using experimental animals has shown that HPA axis activation, induced by restraint stress, increased the growth of the tubercle bacillus (Zwilling et al., 1990). Adrenalectomy and treatment with the glucocorticoid receptor antagonist RU486 abrogated this effect. Furthermore, HPA axis activation suppressed phagocyte function and decreased the capacity of the animals to produce immune augmenting cytokines in response to the mycobacteria (Brown, Sheridan, Pearl, & Zwilling, 1993).

The effects of stress in experimental animals may have important implications in human disease. In an extensive study, tubercular patients were shown to have a dramatic increase in the number of stressful life events approximately 2 years prior to their hospitalization (Homes, Hawkins, Bowerman, Clarke, & Joffe, 1957). Likewise, mortality due to tuberculosis has been shown to be higher in subjects who have experienced divorce (Somers, 1979). It is possible that the reactivation of tuberculosis may be a consequence of suscepti-

ble populations being affected by stressors. Stress, mediated by neuroendocrine-immune interactions, may significantly contribute to this infectious disease, which continues to be a serious health hazard worldwide.

### *Viral Infections*

Colds and influenza have been useful models to evaluate the role of psychoneuro-immunology in human disease. As discussed previously, Cohen et al. (1991) evaluated the significance of psychosocial factors on the common cold. Subjects were inoculated with respiratory viruses, and the risk of developing the infectious disease was directly associated with chronic stress. This study and many others showed similar effects of stress on the development of colds and influenza but no direct effect of stress on the immune system of the more susceptible individuals (Clover, Abell, Becker, Crawford, & Ramsey, 1989).

Many other studies have evaluated the effects of stress on latent viral infections caused by herpes simplex virus, EBV, and HIV. These viruses are typically latent in humans, and the hypothesis that stress favors viral reactivation has been evaluated. These studies have shown that exposure to acute psychological stressors (e.g., examinations and spousal discord) and chronic psychological stressors (e.g., nuclear disaster and caregiving) is associated with high antibody titers to these viruses. These viruses are thought to be controlled by normal host cell-mediated immune response. When stress reduces the cell-mediated immune response, the virus replicates and stimulates an antibody response that is typically non-protective (Kiecolt-Glaser et al., 1991). In the case of genital herpes, Kemeny, Cohen, Zegans, and Conant (1989) showed that a negative mood state was correlated with a decrease in CD8+ lymphocytes (the principal effector against herpesvirus) and herpetic lesion recurrence. Likewise, psychological stress has been shown to predispose an individual to the onset of infectious mononucleosis (Glaser et al., 1991). This work remains

controversial, however, and the role of psychoneuroimmunology in latent viral infections, viral reactivation, and immune stimulation requires further investigation.

### *Fungal Infections*

Although the association between emotional stress and infectious mycological disease has been long suspected, only recently has considerable attention been paid to this association. Fungal infections are well-known to be associated with the stressful conditions of pregnancy, surgical trauma, cancer, organ transplants, long-term antibiotic use, corticosteroid therapy, diabetes mellitus, critical illness, and prematurity (Reszel, Mishra, Mishra, & Pierson, 1993; Shareef, Myers, Nagabhushan, Mathews, & Witek-Janusek, 1998; Witek-Janusek, Cusack, & Mathews, 1998).

Stress hormones such as cortisol and adrenaline are known to enhance pathogenesis of experimental fungal disease (Odds, 1988). For example, *Candida albicans* and related fungi are endogenous opportunists, and infections with these fungi are typically associated with debilitating or predisposing conditions or both. *Candida* infections are the first symptom of active AIDS to appear in HIV-positive individuals. One factor shared by AIDS patients and the other susceptible individuals described previously is hormonal imbalance resulting from HPA axis activation. Furthermore, emotionally affected women who perceive their situation to be stressful have a higher incidence of vaginal *Candida* infections (Reszel et al., 1993). Candidiasis also appears frequently in people undergoing surgery, a unique form of stress that involves emotional stressors (anxiety), chemical stressors (anesthesia), and physical stressors (surgery) (Mishra et al., 1994). Similarly, the emotional stress of divorce has been positively correlated with increased incidence of the carriage of *Candida* (Reszel et al., 1993). Such associations of stress and fungal infection in vulnerable populations are only beginning to be understood.

## ➤ STRESS-IMMUNITY AND NURSING SCIENCE

The holistic view of human nature ascribed to by the discipline of nursing is harmonious with the philosophical underpinnings of psychoneuroimmunology (McCain & Smith, 1994; Zeller, McCain, & Swanson, 1996). As a result, nurse researchers have used a psychoneuroimmunological framework in their research and have made significant contributions to the scientific growth of this field. Nurse investigators have examined stress-immune interactions in a variety of immune-based illnesses, including asthma, HIV, and cancer. In addition, nurse scientists have documented the immunosuppressive nature of postoperative pain and the effects of stress on wound healing. A psychoneuroimmunologic framework has also been used to understand the immunologic implications of child birth and postpartal stress on maternal-infant well-being. Some of these studies are addressed in the following discussion.

Asthmatic symptoms can often be initiated and potentiated by stressful life events. Kang et al. studied the effect of examination stress in asthmatic and nonasthmatic adolescents. Their results revealed that examination stress produces significant alterations in circulating immune cell subsets and in both proliferative and cytolytic activities. No differences were found between the asthmatic and nonasthmatic adolescents, however. Both healthy and asthmatic adolescents reported similar levels of stress and similar changes in immune cell numbers and function (Kang, Coe, Karaszewski, & McCarthy, 1998; Kang, Coe, & McCarthy, 1996). The lack of a relationship between asthma status and social support was believed to be due to the stability and well-managed nature of this asthmatic population (Kang, Coe, McCarthy, & Ershler, 1997). In a similar study, examination stress in adolescent asthmatics produced a bias toward a Th2-like pattern of cytokine production compared to that of nonasthmatic adolescents (Kang, Coe, McCarthy, et al., 1997). These studies are suggestive and need to be

replicated in asthmatics with less stable disease and in naturalistic situations of more intense or chronic stress or both.

The laboratory of Gayle Page conducted a series of compelling experiments in rodents that showed that untreated postoperative pain led to impaired NK cell activity and enhanced tumor metastases (Ben-Eliyahu, Page, Yirmiya, & Shakhar, 1999; Page & Ben-Eliyahu, 1997; Page, Ben-Eliyahu, & Liebeskind, 1994; Page, Ben-Eliyahu, Yirmiya, & Liebeskind, 1993). In her model, rats were subjected to laparotomy and injected with NK cell-sensitive radiolabeled tumor cells that metastasized to the lung. Rats that were treated with morphine, and that exhibited signs of pain relief, had significantly less radiolabeled tumor in the lung, fewer metastatic lesions on the lung, and higher postoperative NK cell activity (Page et al., 1993, 1994). These results suggest that untreated postoperative pain leads to impaired immune function (e.g., reduced NK cell activity) and potentially increased organ localization of tumor emboli. In addition, this research group has demonstrated that the degree of postoperative pain immunosuppression is related to the estrus stage of the rat, suggesting that reproductive hormones may affect the stress-immune response to surgical stress (Ben-Eliyahu, Page, Shakhar, & Taylor, 1996). As a whole, the studies of Page and colleagues indicate that the treatment of pain is necessary not only to alleviate suffering but also to prevent pain-induced immunosuppression and possible tumor metastatic spread. Although these observations have been made in animal models, others have shown in humans that surgery for tumor resection leads to a postoperative reduction in NK cell activity compared to preoperative levels (Pollack, Lotzova, & Stanford, 1992). Evidence that healing and recovery from surgery are potentially altered by stress has also been provided by Wysocki (1996), who found that noise stress delayed wound healing in an animal model. Also, McCarthy, Ouimet, and Daun (1991) have provided evidence that noise stress alters lymphoid cell function needed for tissue repair. The previously discussed results support the

supposition that an individual's psychological state can influence surgical recovery by altering various aspects of immunity (Kiecolt-Glaser, Page, Marucha, MacCullum, & Glaser, 1998).

### *Immunity and HIV Progression*

Living with HIV is replete with multiple stressors, and nurse scientists have contributed to the supposition that the stress-endocrine-immune axis is implicated in HIV disease progression (McCain & Cella, 1995; McCain & Gramling, 1992; Robinson, Matthews, & Witek-Janusek, 1999a; Robinson et al., 1999b). Stress-induced neuroendocrine activation leads to elevations in plasma cortisol. *In vitro*, physiological concentrations of cortisol increase HIV replication in monocyte-derived macrophages, suggesting a potential role for stress hormones in HIV disease activation and progression (Swanson et al., 1998). The effectiveness of stress-reducing interventions in HIV disease has been evaluated by McCain et al. In a pretest-posttest design, the effect of a 6-week stress management program in HIV disease was evaluated (McCain et al., 1996). Outcome measures at 6 weeks and 6 months included perceived stress, quality of life, psychological distress, illness-related uncertainty, and CD4+ T lymphocyte levels. Although the program improved measures of emotional well-being, no significant changes in CD4+ lymphocyte levels were detected. It is likely that CD4+ lymphocyte number may not be a sensitive indicator of improvement in immune function, and other types of immunological assessment may yield more positive results.

In an ongoing intervention study, Robinson and colleagues are examining the efficacy of an 8-week, mindfulness meditation-based stress-reduction program on psychoimmune variables in HIV-positive individuals. These investigators are measuring NK cell activity, which is an important host defense mechanism against viral infections, and opportunistic microbial infections, which cause significant morbidity and mortality in HIV-positive

patients. Preliminary data from the study suggest a positive effect of this program on psychological well-being and immune status (Robinson et al., 1999a). Undoubtedly, nurse investigators will continue to explore the role of stress in HIV disease management and progression.

Stress-induced immunosuppression may have special relevance to the nursing care of cancer patients who undergo immunosuppressive therapies. The emotional stress of undergoing breast biopsy for cancer diagnosis has been clearly demonstrated and presents a useful human paradigm to study psychologic stress (Hooper, Mathews, & Witek-Janusek, 1997; Witek-Janusek & Mathews, 1999b). Anticipation of breast cancer diagnosis has been shown to alter immune cell subsets (Fillion, Lemyre, Mandeville, & Piche, 1996) and TH1 and TH2 cytokine production (Witek-Janusek & Mathews, 1999b). The effect of stress on gene transcription factors has been investigated in women undergoing diagnostic breast biopsy. Gene transcription factors play a significant role in the immune response and can regulate the production of cytokines (Wulczyn, Krappmann, & Scheidereit, 1996). In the diagnostic breast biopsy paradigm, nuclear localization in lymphocytes of two transcription factors, NF- $\kappa$ B and AP-1, was decreased in women experiencing significant emotional stress, whereas when stress was relieved (post-biopsy) the nuclear localization of these gene transcription factors was similar to those of age-matched control women (Nagabhushan, Mathews, & Witek-Janusek, 2000). If and how these factors relate to the modification of immune function and to cancer outcome remain to be determined, but such studies will move this field to a molecular understanding of the effects of stress.

Nurse researchers have used a psychoneuroimmunologic approach toward understanding the impact of childbirth and postpartal stress on maternal child health. Maureen Groer and her research team at the University of Tennessee College of Nursing have demonstrated that childbirth stress leads to a reduction in maternal secretory immuno-

globulin A (sIgA). This reduction was most pronounced in women who reported an increased state of anxiety. Women with very low or undetectable levels of sIgA had a greater incidence of postpartal complications, and their infants had more illnesses. These results indicate that the stress of childbirth can have profound effects on maternal immune function, which can alter the clinical course of mothers and that of their infants (Annie & Groer, 1991). Interestingly, Groer, Mozingo, et al. (1994) demonstrated that touch, provided by a 10-minute slow-stroke effleurage back rub, was shown to increase sIgA levels in elderly adults. It remains to be determined if such an intervention may blunt the decrease in sIgA observed during parturition. Other nurse investigators have demonstrated that glucocorticoid hormones can profoundly influence the pattern of cytokine production (i.e., colony-stimulating factors) from neonatal mononuclear cells obtained from umbilical cord blood. Such immunomodulation may alter the newborn's ability to resist infectious pathogens (Witek-Janusek & Mathews, 1999b).

The unique psychologic and immunologic relationship between a breast-feeding mother and her infant is an intriguing paradigm in which to evaluate the stress-immune relationship. Stress-induced alterations in maternal immunity in breast-feeding mothers could potentially alter their capability to provide optimal levels of immunoglobulins for their infants. Postpartal mothers of preterm infants report high levels of mood disturbance compared to the general population. Mothers who score higher on negative mood subscales of the Profile of Mood States produce less milk sIgA than those who report lower negative mood states. Conversely, mothers who report higher vigor and anger produce greater amounts of milk sIgA (Groer, Droppleman, & Mozingo, 1999). Interestingly, an inverse relationship between cortisol levels and sIgA in breast milk has been reported, such that the higher milk cortisol was associated with lower milk sIgA. It is plausible that increased maternal stress leads to elevated plasma and milk cortisol. Higher levels of milk cortisol may

impair milk immune cell production of immunoglobulins or other immune cell functions or both (Groer, Humenick, & Hill, 1994; Groer et al., 1999). Such stress-induced alterations in milk endocrine and immune composition may potentially impact the immunologic benefits that infants receive from breast milk and certainly require additional investigation. This is especially relevant to premature and low-birth-weight infants, who are at high risk for infectious illness.

### ► FUTURE DIRECTIONS AND NURSING IMPLICATIONS

The guiding premise of psychoneuroimmunology is that stress-induced impairment of immune function influences disease progression or response to therapy or both. These types of investigations are directed toward an understanding of the effect of the psychoendocrine stress response on the immune system, particularly within the context of cancer, autoimmune disease, infectious disease, and maternal child health. Nurses must recognize the potential effectiveness of biobehavioral approaches to the care of patients with immune-based disease. Such approaches to stress management may not only improve the quality of life and emotional well-being of targeted populations but also halt disease progression or complications from opportunistic infection or both.

Complementary and alternative therapies integrate preventive and curative therapies that consider the whole person and are used to "complement" traditional approaches to illness. The use of complementary and alternative therapies by American health care consumers has markedly increased; rigorous scientific testing of such practices has lagged behind, however (Eisenberg et al., 1998; Fontanarosa & Lundberg, 1998). Increased use of massage, touch, meditation, acupuncture, yoga, botanical herbs, guided imagery, and behavioral-based stress reduction programs has spurred a renewed interest in understanding the scientific basis for such ap-

proaches toward healing and health maintenance. This integrative biobehavioral, mind-body, therapeutic approach is harmonious with the view of psychoneuroimmunology and that of nursing science.

As discussed previously, the links among one's emotional state, neuroendocrine activity, and immune response are well described. Future emphasis needs to be placed on understanding the mechanism(s) of stress-induced immune dysregulation and the relationship between stress-induced immune dysregulation and health outcome. That is, do stress-induced changes in immunity alter health, and can stress-reducing interventions that strengthen immunity halt disease progression and improve health? These are critical questions that require intensive empirical investigations using human paradigms of stress. Such approaches will lead to a better understanding of disease and to better diagnosis, treatment, or both of stress-induced immune dysfunction. The results will provide the scientific foundation that will lead to the identification of individuals "at risk" for psychological distress and altered immune reactivity. Such identification will permit the development of psychologically based interventions designed to reduce stress, promote immunocompetence, and hence improve health. Such interventions may prove to be cost-effective additions to traditional forms of treatment or therapy or both and hold promise for disease control. Ultimately, this will serve to enhance the quality and the quantity of life.

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# Issues in the Design and Implementation of Psychoneuroimmunology Research

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*Research that uses a psychoneuroimmunology (PNI) framework seeks to determine whether valid associations exist among stress, immune function, and health. These associations are difficult to conclusively determine due to the fact that PNI research is fraught with methodological difficulties. These difficulties arise from the multifaceted and complex nature of the neuro-endocrine-immune network that is the phenomenon of interest in PNI. This article discusses multiple issues of which investigators should be aware when designing and implementing PNI research including (1) the control of potentially immunomodulating variables related to demographics, behavior, and lifestyle; (2) the manner in which stress, endocrine function, immunity, and health outcomes are measured in consideration of the theoretical relevance to the research question, population, or disease entity under study; (3) the way physiological specimens are procured and stored; and (4) the methods by which assays are performed.*

**Key words:** psychoneuroimmunology, methodology, measurement, stress, health outcomes, immunity

**P** psychoneuroimmunology (PNI) refers to the study of the interactions among behavioral, neural, endocrine (or neuroendocrine), and immunological processes of adaptation (Ader and others 1995). Specifically, PNI is concerned with the bidirectional communication among various parts of the central nervous, endocrine, and immune systems (Rabin and others 1989). PNI explicates the pathways and mecha-

nism by which psychological stimuli or stress influences physiology and health. In essence, PNI research is the "science" that explains the connection between mind and body.

A major goal of PNI research is to determine whether valid associations exist among stress, immune function, and health. These associations are difficult to conclusively determine due to the fact that PNI research is fraught with methodological difficulties. These difficulties arise from the multifaceted and complex nature of the neuro-endocrine-immune network that is the phenomenon of interest in PNI. This article addresses a number of methodological issues of concern in the design and implementation of PNI research studies.

Hypotheses in PNI research generally relate to the ability of psychological factors such as stress to account for variances in neuroendocrine, immune, and health-related dependent measures. The number of potential variables that could affect the dependent variables under investigation confounds establishing such a relationship. This issue is most salient in relation to immune function due to a large number of potentially

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immunomodulating variables. Several types of potential confounding are discussed in this section. In general, strategies to deal with these extraneous variables include exclusion from study or matching/stratifying subjects among groups according to the variable or use of multivariate analysis (Kiecolt-Glaser and Glaser 1988; Zeller and others 1996).

### Demographic Variables

#### Gender

The majority of PNI research has been conducted solely on men. Comparisons between genders are difficult due to the differential effects of gonadal hormones on immune function (Goto and Nishioka 1989; Marchetti and others 2001). Complicating the inclusion of women in PNI studies is the cyclical variation in hormone secretion across the menstrual cycle. For example, natural killer cell activity (NKCA) has been shown to differ significantly across 3 menstrual phases, with greatest activity in postmenses and least activity in menses (Ben-Eliyahu and Shakhar 2001). When women are included in studies, samples should be obtained from them during the same stage of their menstrual cycle (Zeller and others 1996). However, this is often not possible when issues with regard to timing of assessments are integral to the study design. Minimally, menstrual cycle should be assessed, and it should be shown that subjects are randomly distributed or that variance is statistically partialled related to differences in menstrual phase (Kiecolt-Glaser and Glaser 1988). The ratio of estrogen to progesterone is an easy and reliable method of determining menstrual cycle phase.

#### Age and Ethnicity

Age and ethnicity are other potential confounding variables. All aging-related immune changes tend to be in a negative direction including decreased T-cell proliferation and immunoglobulin production (Solomon and Morley 2001). Natural killer cells either increase in number or show no change with age (Solomon and Morley 2001). However, physiological age may not be identical to chronological age (Rabin

1999), so age-related changes in immune function may be related to lifestyle issues as opposed to age *per se*. Differences among ethnic groups are more difficult to characterize due to the paucity of PNI research conducted on ethnic minorities. Still, because genetic differences and differences in susceptibility to various disease states exist among ethnic groups, it may be reasonable to assume that stress and immune response differ as well. It is also possible that lifestyle issues may account for differences among ethnic groups.

### Behavior and Lifestyle Variables

#### Substance Use

Substance use or abuse can greatly confound the results of PNI studies. Substance use has direct effects on immune function (Jaffe 1980), as well as indirect effects via alterations in nutrition (Chandra and Newberne 1977; Conn 1996) and deleterious health-related behaviors. Alcohol (Jerrells and others 1988; Hosseini and others 2001) and recreational drugs (Donahoe and Faleck 1988; Klein and others 2001) have been shown to have immunosuppressive effects particularly with regard to Th1 cytokines and cell-mediated immune parameters (Hosseini and others 2001). Thus, if an individual is abusing drugs or alcohol, it is probably not possible to separate the immunological consequences of substance abuse from any psychological mediation of immune function (Kiecolt-Glaser and Glaser 1988). Furthermore, it is not clear what level of abuse will have significant immunological consequences (Kiecolt-Glaser and Glaser 1988).

Cigarette smoking can significantly affect the endocrine system and also appears to have adverse effects on some immune functions (Kiecolt-Glaser and Glaser 1988). Another commonly used substance, caffeine, has clear effects on catecholamine secretion and, thus, immune function (Dews 1984). Given the potential confounds of substance use/abuse, it is prudent to conduct a comprehensive substance use history in all PNI study participants and incorporate appropriate control for these variables within the study design.

## Nutrition

Poor nutrition is associated with a variety of immunological impairments including cell mediated and humoral immunity and phagocyte function (Dowd and Heatley 1984; Conn 1996). In addition, distressed individuals often have appetite disturbances that can affect nutrition, so relationships between stress and immune function could potentially be a function of underlying nutritional deficits (Kiecolt-Glaser and Glaser 1988).

Therefore, an assessment of nutritional adequacy should be included for all participants in PNI research. At the minimum, this assessment could include history of recent weight changes or inclusion of a prospective food diary (Kiecolt-Glaser and Glaser 1988). The inclusion of biochemical nutritional assays would provide the greatest assurance of nutritional adequacy in subjects. In particular, serum albumin levels are used as a marker for global nutritional status in PNI research because protein malnutrition is associated with increased frequency and severity of infection (Chandra and Newberne 1977). Other assays used in PNI studies include total iron binding and transferrin (Kiecolt-Glaser and Glaser 1988).

## Sleep

There is evidence that severe sleep deprivation can suppress immune function and lead to infectious illness (Krueger and others 2001). Information on the effect of less severe sleep alterations on immunity is limited (Kiecolt-Glaser and Glaser 1988). Immunomodulatory products, such as the cytokine interleukin-1 (IL-1), also function in the initiation and maintenance of sleep (Kryger and others 1994). Likewise, it has been shown that disruptions in quantity and quality of sleep affect the production of IL-1 (Krueger and others 2001). Additionally, due to the circadian variation of multiple immunomodulatory substances and the immune system itself (Sothorn and Roitman-Johnson 2001), it is necessary to assess whether subjects are day or night sleepers (due to shift-work). The simplest and most accurate means to assess for differences in sleep patterns is a prospective sleep diary. Alternatively, actigraphy (usually a wrist-watch-like monitor) is an accurate and relatively inexpensive means to as-

sess a number of sleep-related variables (Sadeh and others 1995).

## Exercise

Strong and consistent relationships have been found between physical exercise and immune function (Hoffman-Goetz and Pedersen 1994). Evidence suggests that lymphocyte proliferation, NKCA, and lymphocyte numbers are all affected by exercise (Cannon 1996; Nieman 1996). Exercise is generally considered immunoenhancing, but strenuous exercise, like that performed by endurance athletes, has been shown to depress multiple immune functions (Nieman 1996). The immunoregulatory effects of exercise are most likely mediated through neuroendocrine changes, as exercise has been shown to increase secretion of catecholamines, growth hormone,  $\beta$ -endorphins, and adrenal hormones (Hoffman-Goetz and Pedersen 1994). In addition, evidence suggests that exercise can alter cytokine secretion such as increases in IL-1, IL-6, and tumor necrosis factor- $\alpha$  (Hoffman-Goetz and Pedersen 1994), leading to changes in immune function.

Because of the multiple endocrine and immune effects of exercise, it is necessary to account for the variance in dependent measures that is due to exercise in PNI studies. An expensive and complex assessment method is the maximal oxygen uptake. This is a measure of aerobic fitness that is performed during exercise (Kjaer and Dela 1996). Alternatively, exercise histories can be obtained, but these do not account for the physiological effects of exercise or the possible inaccuracy of individuals' reports.

## Medication Use

Multiple medications have endocrine and/or immune actions as a therapeutic or side effect. For this reason, the inclusion of participants in PNI studies who are taking drugs with obvious immunomodulatory effects, such as corticosteroids, antineoplastics, and hormone replacement, should be carefully considered. Another commonly prescribed class of drugs, beta-blockers, has been shown to adversely affect lymphocyte proliferation (Goodwin and others 1979). The endocrine and immune effects of many

drugs and drug interactions remain unknown. A thorough medication history including time of last dosage should be taken on all participants in PNI studies.

Furthermore, a recent survey (Eisenberg and others 1998) estimates a high prevalence of herbal and nutritional supplement use in the U.S. population. Many of these supplements are taken for their presumed immunoenhancing effect. Evidence-based research data are lacking on the mechanism of action and clinical effects of many of the most widely used supplements. PNI investigators should include assessment of herbal and nutritional supplements in the design of studies.

### Health Status

The immune system will become activated upon challenge by a foreign antigen. Infectious illness may alter many of the immune parameters of interest in PNI studies. For example, individuals who currently have (or have recently had) an episode of viral disease show alterations in natural killer cell activity and lymphocyte proliferation (Lumio and others 1983). For PNI research, it is recommended that immune data not be gathered on individuals who have reported an infectious illness within the past 2 weeks (Kiecolt-Glaser and Glaser 1988).

Immune-mediated chronic diseases, such as HIV, cancer, and autoimmune processes, cause multiple alterations in immune function and make interpretation of PNI study results difficult. However, individuals with these chronic conditions are of interest to PNI investigators in terms of exploring the effects of stress on disease progression. Therefore, it is necessary when studying individuals with immune-mediated diseases to control for factors related to the pathogenesis of the disease, such as length of illness and disease stage and factors related to immune-targeted therapies.

### Variable Measurement Choice

A critical step in the planning of PNI research studies is the appropriate selection of variables and methods to measure the variables. Research that uses the PNI framework would require measurement of stress, neuroendocrine activation, immune function, and health outcomes. At the minimum, a PNI research study would attempt to measure 2 of these phenomena.

There is a vast array of variables that could represent the phenomena of interest in PNI. Therefore, the rationale for choice of variables and the means by which they are measured should be clearly explicated in all PNI studies.

### Stress

Stress measurement has developed via a multitude of operational formats due to a wide array of stress theories. Self-report is the primary modality of stress measurement in PNI because self-report of stress accounts for the cognitive processes central to many definitions of stress. Few self-report stress measures were developed through strategies that began with a theoretical definition of stress. Rather, most have been adapted as stress measures because the concept(s) they operationalized were judged to represent, *a posteriori*, acceptable definitions of stress (Derogatis and Coons 1993).

A review of the PNI literature indicates that most investigators use multiple measures of stress within a given study. Such an approach is prudent given the complex nature of stress and the lack of consensus on a definitive surrogate measure for stress. However, multiple self-report tools can introduce a burden on participants in terms of time commitment for study participation. This is especially true when the subjects under study are ill and lack the energy and stamina to complete multiple psychometric tools. Investigators must carefully consider the value of each self-report tool used in light of the burden placed on subjects and the potential role that burden could play in subject attrition and incomplete data. In general, investigators should consider deleting 1 or more highly correlated measures.

Simply enumerating the psychological test instruments that have been used to operationalize stress represents an encyclopedic task (Derogatis and Coons 1993). Therefore, discussion will be limited to broad categories of stress measures. Many PNI studies include a stress measure that assesses the number and impact of events that have been shown to be significant stressors. These instruments are generally labeled as life event measures and are consistent with a stimulus-oriented conceptualization of stress. A large number of empirical studies have demonstrated correlations between adverse life events and various indices of health (Derogatis and Coons 1993). Furthermore,

life event measures tend to be less affected by factors such as response bias and distortion of memory (Derogatis and Coons 1993).

Response-oriented measures of stress are also prevalent in PNI research. By a modest estimate, hundreds of self-report measures have been developed to address the various domains of psychopathology, mood and affect, psychological adjustment, and social adjustment that could conceivably meet the basic requirements of a response-oriented definition of stress (Derogatis and Coons 1993). The classes of self-report measures that have been used most often in PNI research as presumptive measures of stress are psychological symptom inventories and scales that reflect mood and affect. Traditionally, most of these instruments have been multidimensional, reflecting the multiple symptom complexes and dysphoric emotions typically invoked to define stress (Piotrowski and Lubin 1990). However, specific syndromes, particularly those that have become synonymous with definitions of stress, such as anxiety, have been the basis for unidimensional instruments (Piotrowski and Lubin 1990).

A few self-report stress measures have emerged directly from transactional stress theory (Derogatis and Coons 1993). By comparison, these measures are relatively new and do not seem to be frequently used in PNI research. Perhaps this is because stress measures are chosen either by investigators, whose emphasis is on the physiological measures, so stress theory is ignored, or by psychologists, who are entrenched in models that emphasize psychopathology. Transactional stress instruments that measure the level of environmental stressors to which the individual is subjected, behaviors that are used to magnify or reduce the impact of the stressor, and the level of conscious emotional distress the individual is experiencing as a result of the stressor-mediator interaction will be of greatest utility to the future of PNI research.

### Neuroendocrine Activation

The inclusion of measures of neuroendocrine activation is a crucial component of PNI research. Much PNI research is limited by the measurement of immune parameters as the only physiological dependent variable. Without measurement of changes in neuroendocrine function, the mechanisms to attribute

changes in immune function remain unknown. However, given the number of neuropeptides and hormones that affect immune function, it is impossible to measure all mediating neuroendocrine substances in any given study. Still, the inclusion of 1 or more measures of neuroendocrine activation, as theoretically appropriate to the study, strengthens the design of PNI studies by allowing investigators to explain observed relationships between stress and immunity via neuroendocrine pathways.

The hallmark of the physiological stress response is activation of the hypothalamic pituitary adrenal (HPA) axis and sympathetic nervous system (SNS); thus, neuroendocrine activation is most commonly measured via cortisol or catecholamine levels in plasma, urine, or saliva. Plasma measurement is regarded as the most precise measure of stress-induced neuroendocrine activation (Axelrod and Reisine 1984). However, catecholamines have short half-lives, so timing of sampling is critical (Dimsdale and Moss 1980). In addition, venous plasma samples may reflect local tissue production and removal of catecholamines and not stress-related changes (Goldstein and others 1987). Investigators should also be aware that the fear associated with venipuncture among some subjects could account for increases in neuroendocrine indices (Schulz and Schulz 1992).

Urinary measurements are of limited usefulness for investigating the short-term responses to stressors in that they measure mean neuroendocrine activity over an interval of time (Dimsdale and others 1987). In addition, urinary clearance of metabolites is influenced by sympathetic activity. Thus, differences in urine metabolites among individuals experiencing different levels of stress may be due to sympathetic arousal instead of changes in the substance being measured (Dimsdale and others 1987). Twenty-four-hour urine collection for measurement ensures less variability than is inherent in 1-time plasma or saliva sampling. The diligence required of subjects for 24-h urine collection introduces a significant possibility of error. As such, reducing collection time to 15 h with appropriate adjustment in interpretation may increase accuracy and compliance. Urine sampling may be more acceptable to potential subjects than plasma sampling due to the noninvasive nature of the sampling procedure. An additional caveat to urine sampling is that secretion of urine metabolites is related to renal function; thus,

baseline creatinine clearance should be measured on all subjects when this sampling method is employed.

The assessment of hormones in saliva may have some advantages for PNI research. The sampling and storage is noninvasive and can be performed by subjects themselves (Biondi and Picardi 1999). Therefore, research designs can employ many more sampling time points than could be done with plasma sampling. In addition, this sampling method may facilitate the execution of field studies (Biondi and Picardi 1999), and be more appropriate in pediatric populations where venipuncture is unduly stressful and urine collection is difficult.

Another concern related to measurement of neuroendocrine activation relates to the manner in which baseline HPA and SNS activity is defined (Rabin 1999). This concern also relates to the definition of baseline immune function. For example, measurements taken while a subject is at rest versus going about his or her daily activities may be significantly different. Because baseline function of the HPA axis and SNS cannot be easily defined or established in PNI studies, baseline immune function becomes a semantic situation, possibly relating to whatever HPA and SNS conditions exist at the time of baseline measurements (Rabin 1999).

### Immune Function

A main argument against the relevance of PNI studies is that it is questionable whether the immunological parameters that are used validly reflect "immuno-competence" (Schulz and Schulz 1992). Measurement of immune function in humans is hampered by a significant sampling problem: the only immune compartment easily studied is the blood. Immune cells in the peripheral blood constitute only 5% of the total pool of immunocytes (Schulz and Schulz 1992). Immune function in the tissues could be opposite of that in peripheral blood depending on the microenvironments (Cohen 1987). Furthermore, PNI research relies heavily on *in vitro* measures of immune function, which further obscures the role complex microenvironments may play in immune function.

Another limitation of immune function measurement in PNI studies is that data are only available for what is studied (Rabin 1999). For example, studying changes in natural killer cell function does not let the

investigator know of other immune changes that were also introduced. With the vast complexity and redundancy of the immune system, a change in one compartment can have an impact on the function of many other components. Thus, reliance on a single immune assay is cautioned. It is not known whether multiple abnormalities of immune function act additively or synergistically to alter susceptibility to immune-mediated disease. If a stressor alters more than 1 immune function, even though each immune function by itself may not alter disease susceptibility, there may be increased susceptibility even if each parameter were not moved out of the normal range (Rabin 1999). Rabin (1999) has suggested that the only immune parameters that are reflective of a summation of the activity of all the immune compartments for either humoral or cellular immunity are the amount of serum antibody to an antigen and delayed hypersensitivity skin reaction. Studies that perform comprehensive evaluations of changes in all immune parameters produced by stress are impossible from a practicality standpoint. Therefore, PNI investigators need to choose immune measures that are theoretically relevant to the question, population, or disease entity under study.

At the single-parameter level, the most frequently used immune measures that yield hypothesis-confirming results are lymphocyte stimulation with a mitogen and NKCA (Schulz and Schulz 1992). Due to the prevalence of these 2 assays in the PNI literature, a brief critique of each will be presented. First, in relation to lymphocyte stimulation, mitogens stimulate a variety of immunocytes with different functions. A depressed lymphocyte proliferation could represent an altered lymphocyte distribution or function, or it could represent influences of macrophages or of humoral factors (Schulz and Schulz 1992). Therefore, it is a fairly non-specific test whose clinical relevance is vague. In addition, mitogen stimulation tests produce highly variable results in normal subjects (Sabbe and others 1983). Similarly, there is extreme variability in NKCA, and a subset of healthy individuals has a persistently low NKCA (Levy and others 1989; Moss and others 1989). Natural killer cells are also particularly susceptible to behavioral variables (Schulz and Schulz 1992) and changing values through the procedure of drawing blood (Girgis and others 1988).

Investigators need to be aware of the functional or clinical implications of the immune function tests cho-

sen for study. In other words, statistical significance does not necessarily equate with clinical significance. Two examples will be used to illustrate this point. First, the normal concentration of IgG in an adult is approximately 1000 mg/dL. Two standard deviations below the mean is approximately 600 mg/dL. If a subject has an IgG concentration of 300 mg/dL, which is statistically different from the mean, it may be assumed that clinical problems will occur. However, there is a marked difference between the statistical abnormality and the physiological concentration of IgG that is needed to protect an individual from bacterial infection, which is considered to be approximately 200 mg/dL (Rabin 1999). Lymphocyte function tests also have difficulties with regard to their interpretation. For example, in lymphocyte mitogenic response to phytohemagglutinin, it is appropriate to conclude that at a very low value such as 10,000 counts per minute (cpm), there is a functional deficit in T lymphocytes. However, counts greater than 50,000 cpm, even though below the normal statistical range, are difficult to interpret with regard to whether there is an associated altered susceptibility to disease (Rabin 1999). Even more difficult to interpret are functional implications of values within normal physiological range such as the difference between 100,000 and 300,000 cpm (Rabin 1999). Similar examples could be present for almost every immune parameter measured in PNI studies. Given the problem of drawing functional conclusions from immune measures, investigators should attempt to measure 1 or more health outcomes in the design of their studies.

Because cytokines are the primary regulators of immune response (Maes and others 1998), the inclusion of cytokine measurement in PNI studies provides an additional level of insight into the complex neuro-endocrine-immune network. The understanding of stress-related cytokine changes is a novel and evolving area of PNI research. The inclusion of measures of cytokine production that are theoretically relevant to the research question will continue to play a part in the evolution of PNI.

### Health Outcomes

From a clinical viewpoint, the goal of PNI research is to show valid associations between stress and health. Changes in the neuro-endocrine-immune network are

irrelevant if they do not mediate susceptibility or progression of disease. Unfortunately, the vast majority of PNI research stops short of investigating health outcomes. Perhaps the reason for this is that the monitoring of health outcomes could make the time required to complete PNI studies prohibitively long. To date, there are no convincing epidemiological studies that inform investigators of the time period required between stressful events and disease- or health-related events. For example, the clinical implication of transient and intermittent immune disruptions has not been sufficiently elucidated. Even if a short-term health outcome is not evident, it does not mean that the stage has not been set for a future disease-related event. Furthermore, the lengthy natural history of many diseases of interest to PNI investigators, such as cancer, HIV, and autoimmune processes, make causal relationships difficult to determine.

Health, like stress, lacks conceptual clarity. There are multiple theories that attempt to define health and its related terms such as disease, illness, and wellness. A detailed discussion of these theoretical frameworks will not be attempted here. However, this discussion of health-related measurement is predicated on the assumption that health is both a subjective and an objective phenomenon. Therefore, subjects' perception of health and clinical disease indicators is a valid measure of health outcomes in PNI research. The inclusion of both types of measures in a given study may give the investigator a more complete assessment of the effects of stress.

### Subjective Measures of Health

Subjective evaluation of health by subjects such as through symptom inventories is one manner by which outcomes are measured in PNI. This can be criticized on 2 counts. First, certain individuals are prone to interpret distress in terms of somatic symptoms (Mechanic 1974). Second, with regard to intervention studies, there is considerable support for a placebo effect. That is, if individuals believe in the effectiveness of a treatment, then they may have a meaningful therapeutic response (Roberts 1995). The inference of both of these criticisms is that subjective evaluation is not valid because subjects may be reporting symptomatology (or relief thereof) that is not "real." Such an inference, however, violates a basic premise of PNI,

which is that mind and body are inextricably linked within a unitary system.

Within PNI, a symptom is a symptom and an effect is an effect. For example, with regard to somatization, if a subject reports myalgia, it is irrelevant from the PNI perspective if the cause of the myalgia is infection or emotional distress. Physiologically, both alterations could cause the release of pro-inflammatory cytokines to induce the symptom. In either case, the symptom is "real." As for the placebo effect, if an individual can alleviate pain through cognitive restructuring of information, then he or she has likely activated a physiologic mechanism, such as release of endogenous opiates, resulting in a "real" analgesic affect. The sum of the argument is that terms such as somatic and placebo negate the premises of the PNI framework by relegating certain outcomes to the effects of mind-only, which negates the existence of the neuro-endocrine-immune network. Therefore, the inclusion of subjective measures of health outcomes in PNI research provides investigators with more complete information on the effects of stress (or stress reduction) than would be possible with solely objective measures of health status.

### Objective Measures of Health

Objective measures of health status, such as incidence of infectious illness, are important in the design of PNI studies because their inclusion is central to meeting the primary goal of PNI research, which is to demonstrate valid associations between stress and susceptibility and progression of immune-mediated illness. A typical PNI study hypothesis would state that variance in susceptibility to infectious illness can be accounted for by levels of stress. This hypothesis is predicated on the assumption that variance in infectious illness is a function of host factors (i.e., immune compromise due to stress). However, pathogenic agent factors such as infectivity, virulence, pathogenicity, immunogenicity, time of contagion, and amount of contagion also affect susceptibility and progression of infectious illness (Harkness 1995). Thus, studies of naturally occurring illnesses are confounded by these agent factors that cannot be controlled by the investigator.

The common cold and influenza are 2 useful study models for PNI research (Biondi and Zannino 1997).

The acute course and relative homogeneity of agent factors of these illnesses allow the investigator more confidence in attributing differences in susceptibility and progression to stress. In addition, the benign outcome of these viral infections enables longitudinal studies with experimental inoculation. Such a study design provides precise results on the role of host factors in these illnesses because exposure source, nature, quantity of pathogen, and time of infection are controlled (Biondi and Zannino 1997).

The choice of health outcome measure must be theoretically and biologically related to the chosen measure of immune function within a given study. Reactivation of latent viral infection is a possible quantifiable health outcome for PNI research. Cell-mediated immunity is a critical factor in the control of latent viral infection such as herpes virus (HSV) (Glaser and Gotlieb-Stematsky 1982). A stress-related reduction in cell-mediated immunity is reflected in an increase in specific antibody titer that follows an increased production of viral antigen (Glaser and Gotlieb-Stematsky 1982). Thus, in a PNI study using a HSV model, the health outcome might be appearance of a mucosal lesion that would be explained via measures that show a depression in cell-mediated immunity.

Similarly, wound healing is being used as an objective health outcome in PNI research (Marucha and others 2001). Cellular immunity has an important role in the regulation of tissue repair. More specifically, pro-inflammatory cytokines regulate the ability of fibroblasts and epithelial cells to remodel damaged tissue (Barbul 1990). Thus, if wound healing is used as the health outcome, then pro-inflammatory cytokine measures (or an immune measure reflective of cytokine dysregulation) should be included as an immune function measure.

### Sample Procurement and Processing

PNI investigators need to be aware that the manner in which specimens for physiological measurements are procured and processed can affect results. Neuroendocrine markers, such as cortisol, have a natural diurnal variation (Barden and others 1982; Bateman and others 1989). Significant circadian variation is also seen in immune parameters such as distribution of lymphocytes in peripheral blood and NKCA (Dhabhar and others 1994; Kronfol and others 1997).

Control for these circadian variations is critical in the design of PNI studies. Therefore, samples should be obtained from all subjects within the same 1- to 2-h time period of the day (Zeller and others 1996). If this is not possible, then the time of day of sampling should be recorded for all subjects so that circadian variations can be considered in the interpretation of results.

Another issue is the amount of time between specimen collection and assay. Storage time can have significant effects on some functional immunological parameters (Fletcher and others 1987). Thus, functional analyses should be performed as soon as possible after specimen collection. A reasonable laboratory standard would be to run all analyses within 6 h of specimen procurement. The time lapse between collection and analysis should be recorded for every sample so that it can be referred to as a possible confound in the interpretation of results.

A related issue is the use of fresh or frozen samples for functional immune analysis. Due to the time frame required for the performance of functional analysis and the desire to run multiple samples simultaneously to decrease interassay variability, some laboratories have opted to run functional analysis on frozen cells (Fujiwara and others 1986). However, freezing cells could have significant results on functional measures of immunity (Whiteside and others 1990). Freezing of cells can affect factors that could enhance or depress the functional activity. For example, freezing of cells permanently inactivates monocytes that have a suppressive regulatory role in NKCA. Therefore, functional assays should always be run on fresh samples.

It is prudent to run as many samples simultaneously as possible in order to decrease the interassay variability that is inherent in laboratory analysis. It has been shown that the day on which samples are collected can account for as much as 85% of between-group differences (Kiecolt-Glaser and Glaser 1988). If the laboratory procedure is unaffected by specimen freezing, as is the case with hormonal analysis and cell counts, specimens should be frozen for future simultaneous analysis.

### Laboratory Issues

Investigators should purchase sufficient quantities of all laboratory supplies for an entire study at the beginning of a study (Kiecolt-Glaser and Glaser 1988;

Zeller and others 1996). Differences in agents, media, and even plasticware can have a significant effect on study results. For example, a 10-fold difference in the relative values for  $\gamma$ -interferon was found using different lots of mitogen in 2 studies where lymphocytes were stimulated to produce interferon (Kiecolt-Glaser and Glaser 1988).

In addition, biometric precision (a term analogous to the psychometric concept of reliability) should routinely be evaluated through test-retest procedures both within (intra-assay) and across (interassay) measurement occasions while controlling biological variability (Zeller and others 1996). Test-retest reliability is frequently evaluated in the laboratory through the use of frozen standards and/or fresh normal controls repeated over time (Zeller and others 1996). Furthermore, careful training of technicians and ongoing monitoring of technicians' results can increase biometric precision.

### Conclusions

The complex nature of the phenomena of interest to PNI investigators necessitates consideration of multiple methodological issues in the design, implementation, and interpretation of results of PNI studies. It is clear that the "perfect" PNI study would require unlimited laboratory personnel, unconstrained subject access, and limitless funding (Kiecolt-Glaser and Glaser 1988). The reality for PNI researchers is normally quite different from this ideal scenario. The research question, study population, or study purpose may make some of the methodological issues more or less relevant. Therefore, PNI investigators need to be aware of these issues so they can logically and rationally choose to control that which may most likely affect their particular study. Regardless, investigators need to be able to discuss the limitations of their studies in light of these methodological issues.

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TH1/TH2 CYTOKINE PRODUCTION DURING THE STRESS  
OF BREAST CANCER DIAGNOSIS. L. WITEK-JANUSEK and  
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Psychological stress can lead to changes in immune function. In this study, the experience of diagnostic breast biopsy was evaluated for its effect, as a naturalistic stressor, on NK cell activity (NKCA) and cytokine production. A within group design in which women experiencing heightened psychological stress (pre biopsy) followed by alleviation of stress (post-biopsy) was employed. Psychological stress was measured by assessing mood disturbance, perceived stress, and anxiety. Most women experienced heightened perceived stress, anxiety, anger, depression, and tension prior to biopsy with alleviation post biopsy. Additionally, most women experienced decreased vigor and increased fatigue pre biopsy with alleviation post biopsy. Overall, these psychological effects corresponded with a reduction in NKCA pre-biopsy and an increase in NKCA post biopsy. Women with continued high mood disturbance and high stress post biopsy had even further reductions in NKCA. Women with high mood disturbance and high fatigue also had decreased capacities to produce interferon  $\gamma$ . Further, Th1/Th2 ratios of IL-2 or interferon  $\gamma$  : IL-10 showed that women with heightened perceived stress and fatigue had reduced ratios of Th1 to Th2 production of cytokines. These observations demonstrate this paradigm of human stress to result in not only an acute reduction in immune function at the time of breast biopsy but also a sustained reduction in NKCA and Th1 cytokine production with continued stress.

contribute to a reduction in CNS infiltrates, decreased demyelination, and suppression of EAE clinical signs under conditions of elevated Cort. (Supported by NIH Grants AI43367, AI35960.)

*Cytokine Production and Natural Killer Cell Activity in Women Stressed by Breast Cancer Diagnosis.* Linda Witek-Janusek\*<sup>†</sup> and Herbert L. Mathews,\*<sup>‡</sup> \*Center for Biobehavioral Immunology, <sup>†</sup>Department of Maternal Child Health, Niehoff School of Nursing, and <sup>‡</sup>Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University of Chicago Medical Center, Maywood, Illinois 60153.

Undergoing breast biopsy for cancer diagnosis is an emotional experience that may impair immune function. This study determined the effect of psychological state on cytokine production and natural killer cell activity (NKCA) in women pre- and post-breast biopsy. Perceived stress, anxiety, and mood disturbance were heightened pre-biopsy in women anticipating biopsy of their breast compared to control women not undergoing biopsy. Post breast biopsy, stress, anxiety, and mood disturbance normalized. NKCA was depressed both pre- and postbiopsy compared to nonbiopsied control women. However, postbiopsy NKCA was greater than prebiopsy NKCA in the same women. IL-6 production by peripheral blood mononuclear cells of women in the biopsy group was increased both pre- and postbiopsy compared to control women. Conversely, IFN- $\gamma$ , IL-4, and IL-10 were depressed pre- and postbiopsy relative to control women. No change in IL-2 production was observed. Thus, impending breast biopsy produced stress, anxiety, and mood disturbance, which was relieved postbiopsy. Associated with the experience of biopsy was depressed NKCA and altered cytokine production. These changes in immune function continued to be observed despite the normalization of psychological measures and suggest that biopsy stress has a prolonged effect on immune function. In conclusion, psychological and immune dysregulation begin early in a woman's encounter with breast cancer diagnosis and may influence cancer control.

Presented at:

Era of Hope Meeting, Dept. of Defense, Atlanta, GA

June, 2000.

## **PSYCHO-ENDOCRINE-IMMUNE PROFILE: IMPLICATIONS FOR QUALITY OF LIFE IN BREAST CANCER PATIENTS**

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Breast biopsy is an emotional experience and may impair anti-tumor immune responses. This study determined a woman's psycho-immune response pre (T1 and T2) and post (T3 and T4) breast biopsy. Stress, anxiety, and mood disturbance were heightened pre-biopsy in both benign and malignant patient groups. Post-biopsy, stress, anxiety, and mood disturbance normalized in the benign group but remained elevated in the malignant group. Natural killer cell activity (NKCA), a form of anti-tumor immune response, was depressed (T1-T4) in benign and malignant groups compared to non-biopsied control women. At T4, NKCA was lower in the malignant group compared to the benign group. IL-2, a Th1 cytokine, and IL-6, a proinflammatory cytokine, were increased from T1-T4 compared to control women. Conversely, IFN $\gamma$ , a Th1 cytokine, and IL-4 and IL-10, Th2 cytokines, were depressed (T1-T4) relative to control women. Thus, breast biopsy produced stress, anxiety, and mood disturbance, which was relieved post-biopsy in women with benign results but sustained in women with malignancy. Associated with the stress of biopsy was depressed NKCA and altered cytokine production. In conclusion, psycho-immune dysregulation begins early in a woman's encounter with breast cancer and may influence cancer control. Thus, women may benefit psychologically and immunologically by stress-reducing interventions provided during the initial period of cancer diagnosis.

The U.S. Army Medical Research and Materiel Command under DAMD 17-98-1-8120 supported this work.

**Abstract from the Annual Meeting of the Psychoneuroimmunology Research Society, Cape Fear-Wilmington, North Carolina, June 2000.**

Herbert L. Mathews,<sup>1</sup> Moolky Nagabhushan,<sup>1,2</sup> and Linda Witek-Janusek.<sup>2</sup> Aberrant nuclear expression of AP-1 and NFkB in lymphocytes of women stressed by the experience of breast biopsy. *Brain Behavior and Immunity* 14: 114, 2000.

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We have investigated the expression of AP-1 and NFkB in peripheral blood lymphocytes of women scheduled for breast biopsy. Samples were collected when women were informed of the need for biopsy (pre biopsy - T1, 5-7 days prior to the actual biopsy) and 7-10 days after they learned the result of their biopsy (post biopsy - T2). At the time of blood collection, psychological stress was evaluated using Spielberger's State Trait Anxiety Inventory (STAI) and the Profile of Mood States (POMS). Women scheduled to undergo breast biopsy reported significant increases in anxiety (STAI) and mood disturbance (POMS). Gel shift mobility assays showed that mitogen stimulated peripheral blood lymphocytes of these women were less capable of the nuclear expression of AP-1 or NFkB at T1. Similar assessments, 7-10 days after the women learned of the results of their breast biopsy, showed these same women to have a marked reduction in anxiety and mood disturbance and an increased nuclear translocation of AP-1 and NFkB. These results show a significant decrease in nuclear AP-1 and NFkB expression during the period of emotional distress prior to biopsy with a return of nuclear transcription activity to normal levels when distress was relieved. Several studies have correlated increased psychological stress with decreased immune function. The results of this study suggest that psychological stress may mediate immunosuppression by altering the expression of the transcription factors, AP-1 and NFkB.

## Bridging the Gap Between Mind and Body: Psychoneuroimmunology Research

Presented at the Annual Ruth K. Palmer Research Presentation, March, 2002

Loyola University of Chicago

Linda Janusek, Ph.D., R.N. and Matthew Sorenson, Ph.D., R.N.

For centuries, well before the dawn of modern science, the relationship between emotions and health has captivated scientists. Despite this well-traveled historical path, embodying emotions within the science of immunology remained elusive. Only in the last 20 years has the impact of emotions on the immune response and immune-based disease come to be appreciated and accepted in scientific circles. The discovery of clear biological links that allow reciprocal communication among nervous, endocrine, and immune systems fueled the renaissance that led to the evolution of the multidisciplinary field of psychoneuroimmunology.

Psychoneuroimmunology research, which explores the impact of stress and emotions on health, has clear relevance to the discipline of nursing. In our laboratory, two human paradigms of psychological stress and immunity have been studied. The first investigation examined the psychological stress of breast cancer diagnosis on immune activity relevant to cancer control. Undergoing the experience of cancer diagnosis heightens stress perception, anxiety, and mood disturbance. The results of this study demonstrate that the psychological stress and negative emotions that accompany breast cancer diagnosis are linked to impaired cell mediated immune response to tumor cells and dysregulation of cytokines important to tumor control. This occurs in both women with benign and malignant breast disease. The second paradigm examined the effect of stress, coping, and illness uncertainty on immune function in individuals with multiple sclerosis (MS). The pathogenesis of MS, an autoimmune disease, is associated with an imbalance of pro- and anti-inflammatory cytokines. The results of this study show significant relationships among stress, coping and the pattern of cytokines produced in individuals with MS. Moreover, these changes in stress and immunity were associated with self-reported disease symptomatology. Collectively, these two paradigms, in which coherent links between stress and immunity were found, provide evidence for the influence of emotions on immunity and the expression of immune-based disease.

Keating, P. Witek-Janusek, L, and Mathews, HL. "Modulation of the Psychological Response to Breast Cancer Diagnosis," Ruth K. Palmer Annual Research Symposium, Loyola University of Chicago, March, 2002.

**Background:** Women undergoing biopsy of the breast for cancer diagnosis experience psychological stress, no matter what the findings, benign or malignant. Considerable evidence suggests a role for negative emotions in disease progression, including cancer. Yet, there is limited information regarding which psychological characteristics serve to modulate or buffer the stress response to breast cancer diagnosis. In other stressful situations, enduring psychological characteristics, such as sense of coherence (SOC) and resilience (RSL), were shown to modify the psycho-physiologic stress response. **Purpose:** The purpose of this study, therefore, was to determine the relationship among a woman's SOC and RSL and her perceived stress and mood in response to breast cancer diagnosis. **Sample:** Women (21-80 yrs) who were scheduled for a breast biopsy were enrolled along with a group of age-matched control women. **Methods:** A secondary analysis of a data-base from an on-going study examining the psycho-endocrine-immune response to breast cancer diagnosis was conducted. Perceived stress, mood disturbance, SOC, and RSL were assessed before breast biopsy and after breast biopsy findings were known. **Findings:** Positive correlations ( $r = 0.640$ ;  $p < .01$ ) were found between SOC and RSL. In contrast, significant negative correlations ( $r = -0.461$ ;  $p < .01$ ) were found between SOC and perceived stress, and between RSL and perceived stress ( $r = -0.263$ ;  $p < .05$ ). Negative correlations ( $r = -0.432$ ;  $p < .01$ ) were found between SOC and total mood disturbance (TMD), and between RSL and TMD ( $r = -0.287$ ;  $p < .01$ ). **Conclusion:** These findings suggest that SOC and RSL may buffer the psychological response to stress and provide preliminary data for the development of an explanatory model, which can guide psychological risk assessment in women undergoing cancer diagnosis. Early identification of psychological risk can target interventions to facilitate adaptation. Future analysis will explore the role of SOC and RSL in adaptation to cancer and quality of life in breast cancer survivors.

#A91. The Psychological Impact of Breast Cancer Diagnosis Produces Prolonged Alterations in TH1/TH2 Cytokines and Natural Killer Cell Activity. Herbert L. Mathews and Linda Janusek, Loyola University of Chicago, U.S.A. *Brain, Behavior, and Immunity*, Vol. 16, No. 2, April 1, 2002 pg. 179.

Biopsy of the breast for cancer diagnosis is an emotional experience, characterized by anxiety and fear. This experience may impair immune responses. This study evaluated a woman's immunological and psychological response at multiple times pre- and post-breast biopsy. Perceived stress, anxiety, and mood disturbance were heightened prebiopsy. Postbiopsy, perceived stress and mood disturbance decreased but did not return to levels reported by nonbiopsied control women. Natural killer (NK) cell activity was depressed both pre- and postbiopsy, when compared to control women. No change in the number of NK cells was observed. Production of INF $\gamma$  was significantly reduced pre- and postbiopsy, while the production of IL-4, IL-6, and IL-10 were significantly increased by the experience of breast biopsy. The immune dysregulation that accompanied breast biopsy was most marked in women with malignant biopsy findings. Moreover, the results show that women who report the highest levels of stress or mood disturbance had the most marked changes in immune function. Thus, increased perceived stress and mood disturbance, as well as immune dysregulation, accompanied breast biopsy and extended beyond the biopsy experience. Therefore, the psychological and immunological impact of biopsy is not transient and persists beyond the actual experience of biopsy. These observations may be of particular relevance to women diagnosed with malignancy since they face additional stressors related to cancer treatment and adaptation to illness.